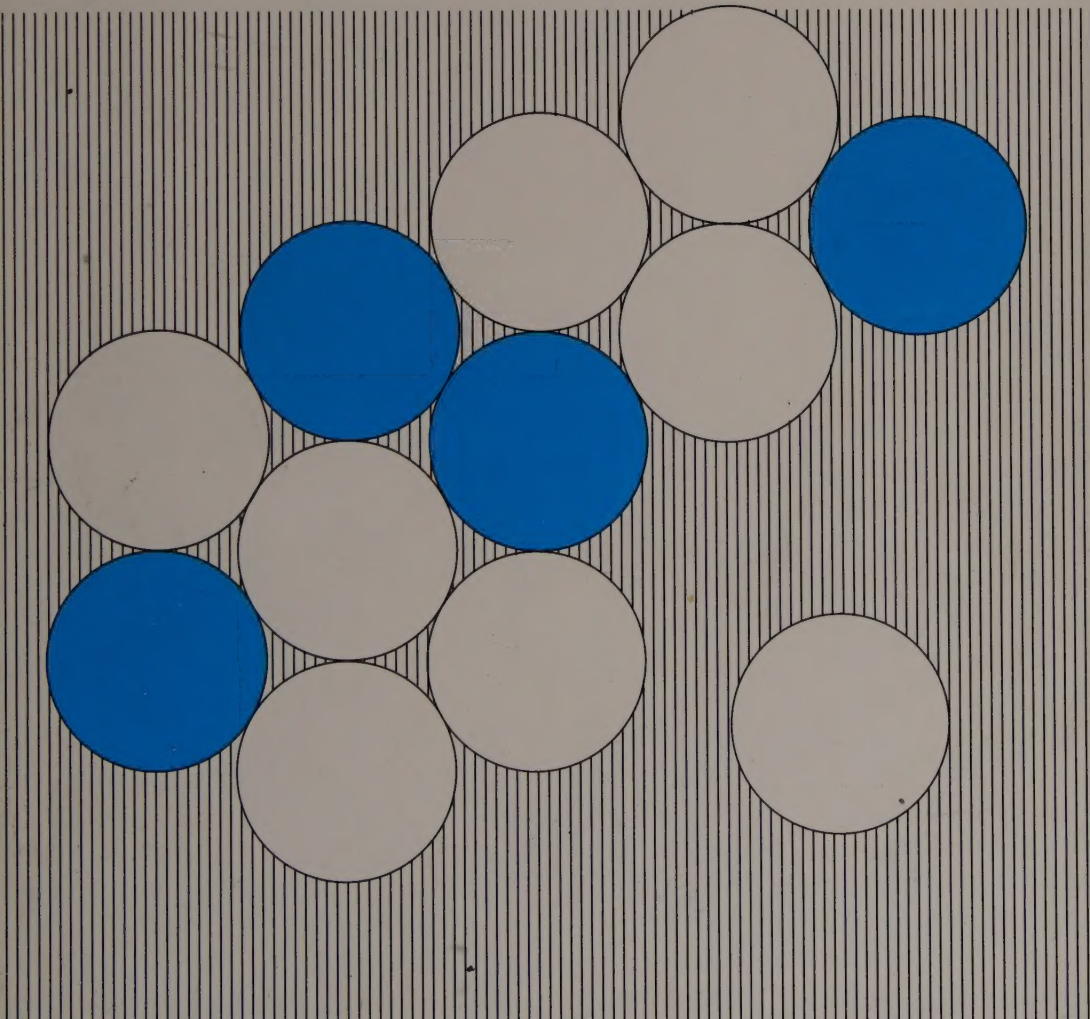
SCIENTIFIC BASIS OF FERTILITY REGULATION

WORLD HEALTH ORGANIZATION

Vaccines for fertility regulation

EDITED BY
G. L. ADA AND P. D. GRIFFIN



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Vaccines for fertility regulation

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SCIENTIFIC BASIS OF FERTILITY REGULATION

Vaccines for fertility regulation

The assessment of their safety and efficacy

Proceedings of a Symposium on Assessing the Safety and Efficacy of Vaccines to Regulate Fertility, convened by the WHO Special Programme of Research, Development and Research Training in Human Reproduction
Geneva, June 1989

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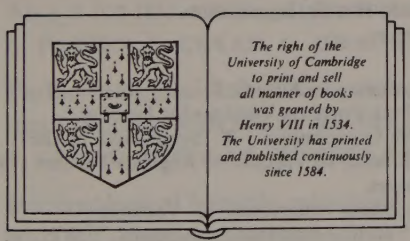

Published on behalf of
the World Health Organization by
CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE
NEW YORK PORT CHESTER
MELBOURNE SYDNEY

FAMILY PLANNING
ASSOCIATION
INFORMATION AND
RESEARCH CENTRE

Published by the Press Syndicate of the University of Cambridge
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
40 West 20th Street, New York, NY10011–4211, USA
10 Stamford Road, Oakleigh, Melbourne 3166, Australia

© World Health Organization 1991

First published 1991

Printed in Great Britain at the University Press, Cambridge

British Library cataloguing in publication data

Vaccines for fertility regulation.

1. Man. Fertility. Regulation. Immunological aspects
I. Ada, G. L. (Gordon Leslie) *1922–* II. Griffin, P. D.
III. World Health Organization
612.6

Library of Congress cataloguing in publication data

Vaccines for fertility regulation : the assessment of their safety and efficacy/edited by G.L. Ada, P.D. Griffin.
p. cm. – (Scientific basis of fertility regulation)
Based on the Symposium on the Assessment of the Safety and Efficacy of Vaccines to Regulate Fertility, held at WHO headquarters in Geneva 12–16 June 1989; convened by the HRP Task Force on Vaccines for Fertility Regulation.
Published on behalf of the World Health Organization.
Includes bibliographical references.
Includes index.

1. Antifertility vaccines–Congresses. I. Ada, G. L.
II. Griffin, P. D. III. HRP Task Force on Vaccines for Fertility Regulation. IV. World Health Organization. V. Symposium on the Assessment of the Safety and Efficacy of Vaccines to Regulate Fertility (1989 : Geneva, Switzerland). VI. Series.
[DNLM: 1. Fertility–drug effects–congresses. 2. Vaccines–immunology–congresses. 3. Vaccines–standards–congresses. WP 565 V116 1989]
RG136.85.V33 1991
615'.766–dc20 90-15076 CIP

ISBN 0 521 39252 7 hardback

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Preface

The WHO Special Programme of Research, Development and Research Training in Human Reproduction (HRP) was established in December 1971. Its activities cover the broad field of research in reproductive health, with special emphasis on the development of new and improved methods of family planning that satisfy the widely varying cultural, religious, personal and service needs of the different populations of the WHO Member States.

A major demand of both the users and providers of family planning services continues to be for methods that provide long periods of effectiveness following a single administration. One way in which this demand might be met is through a novel approach to family planning using antifertility vaccines based on, and directed against, molecules essential for the reproduction process. It has been known for several decades that antibodies can be raised in humans to human spermatozoa and, more recently, that some types of naturally occurring and long-lasting infertility, in both men and women, are associated with the presence of antisperm antibodies. In addition, many investigators have demonstrated that a partial or complete antifertility effect can be induced in experimental animals as a result of immunization with a variety of reproduction-specific molecules.

It was against this background, and in consideration of these issues, that the HRP Task Force on Vaccines for Fertility Regulation was set up in April 1973, with the primary objective of supporting and coordinating studies aimed at the development of safe, effective and acceptable antifertility vaccines. At the time of its formation, the Task Force recognized that, unlike other vaccines which are usually directed against 'foreign' micro-organisms or their products, antifertility vaccines will be directed against 'self' or 'self-like' molecules and represent, therefore, a totally novel avenue of immunoprophylaxis. In addition, antifertility vaccines are not intended to combat life-threatening or debilitating diseases, but are to be used by healthy, fertile individuals who have a number of alternative existing family planning methods from which to choose. These considerations necessitate that the assessment of safety is of paramount importance in the development of these vaccines.

In an attempt to define appropriate studies for evaluating the safety and efficacy of vaccines directed against host components involved in the reproductive process and which could be used for fertility regulation, the Task Force consulted immunologists, toxicologists, immunopathologists, repro-

ductive biologists and representatives of drug regulatory agencies about this issue. The report of these discussions, published in 1978,[1] proved to be a landmark document and, although it was not specifically intended to be so, has served as a set of guidelines for the activities of the Task Force over the past decade. At that time, the following principles for the development of antifertility vaccines were proposed.

(a) To be effective, the target antigen should be essential for the reproductive process.
(b) To minimize the risk of autoimmune disease, the target antigen should be restricted to the intended target.
(c) To minimize the risk of immune-complex disease, the target antigen should not be present continuously in the vaccine recipient but only intermittently and/or at low concentrations.
(d) The antifertility effect of immunization should not be permanent and there should be no demonstrable hazard to offspring born subsequently to vaccine users.

It was recognized that whilst antifertility vaccines could be developed that would be effective at virtually every stage of reproduction, the most acceptable were likely to be those that could prevent fertilization by interfering with gamete interaction, and those that could prevent implantation of the conceptus by disrupting blastocyst development. During the past 16 years, therefore, the Task Force has supported research on vaccines based on sperm antigens, ovum antigens and non-hormonal antigens of the trophectoderm of the preimplantation embryo. Funding constraints in the late 1970s necessitated that the Task Force focus its activities on the most advanced lead at that time, namely an anti-hCG vaccine. This resulted in the development of a vaccine based on the apparently hCG-specific carboxyterminal peptide of beta-hCG (β-hCG–CTP) which was extensively evaluated in a series of preclinical safety and efficacy studies between 1980 and 1984. By 1988, a Phase 1 clinical trial had been satisfactorily completed,[2] and the Task Force is now planning to carry out a limited Phase 2 clinical trial to evaluate the efficacy of this prototype vaccine. Similar studies in each of the above areas have also been supported by a number of other international, national and private funding agencies.

In the 12 years that have elapsed since the publication of the earlier report, a great deal of information has been gathered by HRP and by other programmes, much of which has not been published or otherwise made available to the scientific and medical communities. The Task Force decided, therefore, to convene a Symposium on the Assessment of the Safety and Efficacy of Vaccines to Regulate Fertility in order to discuss these issues and to draw up recommendations for future studies in this area. This symposium was convened at WHO Headquarters in Geneva on 12–16 June, 1989, and was

attended by reproductive biologists, endocrinologists, obstetricians, gynaecologists, immunologists, molecular biologists and pathologists as well as participants with expertise relevant to the social, ethical, legal and political aspects of these vaccines, and representatives of consumer groups, the regulatory authorities, biological standards institutions and the pharmaceutical industry.

Although it was decided that the aims of the symposium should be as broad as possible, in order to cover all aspects of the development and use of antifertility vaccines, the following five general objectives were selected in order to focus the meeting.

(1) To review current information about target antigens for antifertility vaccines and the most effective ways to present them to the immune system.
(2) To review the nature and range of the preclinical testing needed to assess the safety and efficacy of antifertility vaccines.
(3) To discuss and recommend specific requirements for clinical trials of antifertility vaccines.
(4) To foresee and assess the specific technical needs which long-term surveillance of the use of these vaccines would require.
(5) To discuss the social, ethical, legal and regulatory issues which the preparation and use of these vaccines would raise so that the appropriate action could be taken to avoid potential problems in these areas.

In view of the diversity of the subject matter and of the backgrounds and training of the symposium participants, a series of papers was commissioned which reviewed the major areas to be discussed in a manner which could be understood by interested but non-expert readers. These papers, extensively referenced to scientific articles and official documents, are published here together with a report of the consensus reached at the symposium concerning each of these five areas.

Topics as seemingly far removed from one another as vaccination to regulate fertility and vaccination to prevent infectious diseases, share many common facets, such as some of the criteria for the selection of clinical trial volunteers, the performance of clinical trials, and the post-registration surveillance of vaccine use. For these reasons, it is felt that this publication will be of considerable interest not only to those interested in antifertility vaccines but also to the much wider constituency interested in immunoprophylaxis in general.

We should like to acknowledge the secretarial assistance provided by Mrs L. Pasini and Mrs L. Sellaro with the organization and conduct of the symposium and with the preparation of the typescripts of the discussion sessions on which the final report section of this publication is based.

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Welcoming address

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The functions of the World Health Organization are specified in its Constitution and include, *inter alia*: '(a) to act as the directing and coordinating authority on international health work; . . . (c) to assist Governments upon request, in strengthening health services; . . . (n) to promote and conduct research in the field of health; . . . (q) to provide information, counsel and assistance in the field of health.'

To carry out these functions in the field of reproductive health, the organization established, in 1971, the Special Programme of Research, Development and Research Training in Human Reproduction. The mandate of the Special Programme, which is currently co-sponsored by UNDP, UNFPA, the World Bank and WHO, is to promote, coordinate, support, conduct and evaluate research in human reproduction with particular reference to the needs of developing countries.

Since its inception, the Special Programme has been concerned about the inadequacies of currently available contraceptive methods and services as exemplified by the gap between family planning intentions and contraceptive use, by the high discontinuation rates of family planning methods, and by the large number of unplanned pregnancies, many of which end in abortions.

It is broadly accepted that one of the requirements to improve this situation is a greater variety of safe, effective, acceptable and affordable fertility-regulating methods. This is particularly true if the intention is to cater for the needs of our pluralistic world and to be responsive to the wide variety of cultural, religious, social, economic, service and personal factors that influence human reproduction decisions. More and better methods are needed, not only in biomedical terms, but in order to improve the quality of life of human beings. This is the aim of the Special Programme.

There is a clear demand, from both the users and providers of family planning services, for methods that provide long periods of effectiveness following a single administration.

One novel approach which offers particular promise in this regard is the development of antifertility vaccines that would permit immunization against one or more of the molecules specific to, and essential for, the reproduction process; provided such immunization proves to be safe, effective and free of significant and unacceptable immediate side-effects or long-term sequelae.

To investigate the feasibility of this approach, the Special Programme

established, in 1973, a Task Force on Vaccines for Fertility Regulation through which it has supported research on the development of antisperm vaccines, anti-ovum vaccines and vaccines designed to neutralize the biological functions of structural components and hormonal products of the trophectoderm of the early conceptus, such as human chorionic gonadotrophin (hCG).

Although the presence of antisperm antibodies in animals injected with semen and spermatozoa was demonstrated as early as the turn of this century, and an association between antisperm antibodies and some types of infertility was established soon after, it is only during the last 20 to 30 years that the comparatively new science of reproductive immunology has shown that a successful outcome to the reproduction process is dependent on a controlled interaction between the reproductive and immune systems and that gamete fusion and embryo implantation can be interrupted by immunological manipulation.

It is not surprising, therefore, that research on fertility-regulating vaccines has been underway for only a couple of decades, and it is only in the last 10–15 years that this work has reached the stage at which prototype vaccines have been evaluated in animal studies and preliminary clinical trials. The most advanced of these preparations are a variety of prototype hCG vaccines based either on the complete β-subunit of this hormone (as developed at the National Institute of Immunology in New Delhi with the initial support of the Special Programme, and later at the Population Council in New York) or on a synthetic peptide of the carboxyterminal region of β-hCG (as developed by the Special Programme's Task Force). All of these vaccines have undergone, or are undergoing, safety and immunogenicity evaluations in Phase I clinical trials in previously electively sterilized women volunteers, and Phase II clinical trials to assess their efficacy in fertile women volunteers are planned for the early 1990s.

In view of the fact that they are intended for use by healthy individuals, and that they are directed at 'self' or 'self-like' molecules, fertility-regulating vaccines represent a major departure from the more conventional 'anti-disease' vaccines. The programme recognized, at an early stage in its activities, that there was a need to establish guidelines for the preclinical and clinical safety and efficacy evaluation of these novel vaccines and it convened, in 1977, a meeting of immunologists, toxicologists, immunopathologists, reproductive biologists and representatives of drug regulatory authorities to review these issues. The report of this meeting, published in 1978, has served as a set of informal guidelines for the activities of the Task Force ever since, and has been referred to also by other investigators. However, in the 12 years that have elapsed since the publication of that report, our knowledge of the molecular events of reproduction and of normal and abnormal immune processes has greatly increased, and major advances have been made in a number of technologies relevant to vaccine design, development and production. In

addition, many animal and clinical studies have demonstrated the feasibility of developing apparently safe and effective antifertility vaccines based on both natural and synthetic reproduction-specific immunogens.

It seems appropriate, therefore, to convene this symposium now in order that representatives of all of the many disciplines involved in the development, production, monitoring and eventual registration and promotion of antifertility vaccines can discuss the issues and review the challenges to be faced over the next 10 years in the development and use of these preparations.

In this first symposium we will be addressing a wide spectrum of issues, ranging from basic research on vaccine development, through preclinical testing and clinical trials, to the social, ethical, legal and regulatory aspects of such vaccines. In other words, all of the many but necessary steps involved in getting preparations from the laboratory bench to the market place. In view of the wide spectrum of issues to be addressed and discussed, and the correspondingly wide spectrum of expertise represented among the participants, it is likely that it will not be feasible to deal exhaustively with any one of these areas, even in the subgroup meetings. If this is the case, this symposium could be seen as the first of a series with subsequent meetings focusing on one or more of these issues in order that even better defined research strategies and priorities can be drawn up in each of these areas. It is anticipated that this symposium, and the possible subsequent meetings, will be of interest to other WHO programmes and other agencies engaged in vaccine development, either for fertility regulation or for the prevention and control of infectious diseases, and we look forward to increased collaboration with our colleagues in this work.

Our thanks must go the authors, presenters and discussants of the commissioned papers for the substantial efforts they have already made in providing the background documentation for the symposium, and to the chairpersons and rapporteurs who have the primary responsibility for producing the subgroup reports during the course of the symposium.

Your task during this week fits very well, therefore, with some of the constitutional mandates of the Organization. By bringing together the best available expertise to help define regulatory requirements for fertility vaccine development we will be able to provide guidelines that will assist governments in formulating their own regulatory requirements and the international scientific community in defining their activities.

I should like to thank you all for making this possible.

1 Background and objectives

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Introduction

In 1978, the WHO Task Force on Immunological Methods for Fertility Regulation (now the Task Force on Vaccines for Fertility Regulation) discussed the general requirements an antifertility vaccine should meet in order for it to be considered an effective, safe and acceptable method of fertility regulation in humans.[1] Although immunization as a prophylactic measure to combat infectious diseases was widely accepted and practised, there was no precedent to suggest whether or not a vaccine directed against, or cross-reacting with, a self component could be developed and would be both highly effective and safe. There were no guidelines or national or international regulatory requirements to be followed for such a project.

Of the various options available,[2] the Task Force had decided to devote most of its resources to the development of a vaccine directed against the hormone, human chorionic gonadotrophin (hCG). This choice was in part dictated by the fact that the amino acid sequence and the general structure of the hormone were known. This hormone is produced by the peri-implantation embryo and is essential for the establishment and maintenance of early pregnancy. A vaccine directed against this hormone therefore would prevent or disrupt the implantation of the fertilized egg.

Aware of the endocrine disturbances and the increased risk, at least in theory, of autoimmune pathology and other consequences resulting from cross-reactivity of the antibodies resulting from immunization with whole hCG or its beta subunit with human luteinizing hormone (hLH), the Task Force chose to focus attention on the C-terminal 37 amino acid peptide of the beta chain of hCG. The data available at that time suggested this was a unique sequence, at least among human hormones. This approach, it was argued, would prevent the production of antibodies cross-reacting with hLH and greatly reduce the risks mentioned above.

Nevertheless, the Task Force attempted to define the different parameters which needed to be examined and the methodology which might be employed in assessing the safety of this approach. The proposals made at that time, largely on a predictive basis, have served as a valuable guide to the development of hCG and other antifertility vaccines. The C-terminal peptide based hCG vaccine has now successfully undergone a Phase 1 trial in sterilized

women, and a limited efficacy (Phase 2) trial in fertile women is being planned. Other preparations containing the intact beta subunit of the hormone are also being evaluated in clinical trials by other groups. Also, there are new leads with sperm antigens which are closely involved in interactions with ova.

In addition to these specific points, there have been advances in other more general but highly relevant areas. During the 10–15-year development period of these vaccines, a considerable amount of information has accumulated on the immune response to these antigens. Secondly, our understanding of immune mechanisms is much more detailed than previously and this, coupled with improved assay methods, enables a more detailed examination of the immune response to vaccines in general. Thirdly, substantially more is understood about the nature of immunological tolerance which should lead to a reconsideration of the nature of the differences between foreign and self antigens. Fourthly, much more information is now available on autoimmunity and the development of autoimmune disease.

A consideration of all these points led to the decision that the time was now ripe to review and integrate the new concepts and findings. This chapter briefly reviews some of the more general aspects mentioned above and aims to provide background information relevant to the details and discussions in the other chapters. The information obtained during the review will be particularly relevant to other hCG vaccine programmes, and, indeed, should be very useful to any research programme aiming to regulate human fertility by vaccination against a self or self-like antigen. In turn, information from other programmes on aspects such as the impact of antibodies to hLH helps to evaluate possible hazards associated with vaccination against self antigens.

Immune responses to self and non-self antigens

In 1949, Burnet and Fenner[3] proposed that a vertebrate host could distinguish between a self and non-self antigen. One mechanism proposed was the presence of a self marker but this concept was soon abandoned. However, the discovery of major histocompatibility complex antigen (MHC) restriction of thymus-derived (T) cell responses some 25 years later[4] attests to Burnet's remarkable powers of intuition. He went on to propose that the ability to distinguish between self and non-self was acquired by the fetus at or about the time of birth and that introduction of a foreign antigen to the fetus before that time would induce a state of tolerance to that antigen.

This prediction was confirmed by Billingham and his colleagues,[5] thus demonstrating for the first time that there was no intrinsic difference between self and non-self antigens; the essential difference was in the recognition mechanisms of the host.

Recent findings have indeed confirmed this notion, although sometimes the differences are minimal. For example, the major polysaccharide antigen of group B meningococcus is a polymer of N-acetyl neuraminic acid, a sugar that

occurs so widely in vertebrates that the bacterial antigen almost invariably induces only a short-lived, low-affinity IgM response.[6] Sometimes, the occurrence of an autoimmune disease has led to a demonstration of homology between a self and a non-self protein. Thus, it has been known for a long time that infection with group A streptococci can cause rheumatic fever. This is due to cross-reactivity between certain bacterial M proteins and mammalian tissues, particularly cardiac tissues including sarcolemmal membrane proteins and myosin. Recent studies[7] suggest that opsonic antibodies to the bacteria recognize a bacterial 14 amino acid sequence which also occurs in heart tissue.

Cross-reactive epitopes between infectious agents and host components – molecular mimicry – now appear to be relatively common. In one study,[8] up to 3.5% of monoclonal antibodies prepared against 11 viruses were found to react with normal tissues. Data base searches have also demonstrated[9] considerable sequence homology between viral and tissue proteins.

To test the possible significance of one such homology, rabbits were immunized with a decamer from hepatitis B polymerase, which shared 60% homology with the encephalitogenic site of rabbit myelin basic protein. All rabbits developed humoral and cell-mediated immune (CMI) responses to native myelin protein, and several developed pathological lesions in the mid-brain.[10]

It does not necessarily follow that immunization with an antigen which has some homology with a host component(s) will induce the formation of autoantibodies, let alone initiate an autoimmune disease. However, the need for powerful adjuvants and the possible requirements for repeated injections to boost immune responses to subunit or peptide-based vaccines may increase this possibility.

Prevalence of autoantibodies in healthy people

In his Clonal Selection Theory, Burnet[11,12] proposed that tolerance to self antigens involved the deletion of clones of lymphocytes reactive to those antigens. The discovery of autoantibodies shortly afterwards[13] necessitated a revision of this simple concept; surveys carried out at that time on normal populations showed that autoantibodies to a variety of normal body components occur at a high prevalence (approximately 20%) in apparently healthy people.[14] More recently, monoclonal autoantibodies produced from normal mice and human lymphocytes were shown to react with antigens in gastric mucosa, thyroid, anterior pituitary and pancreatic islets.[15]

Other monoclonal antibodies developed from patients with various lymphoplasmocytic diseases reacted with intracellular proteins such as dsDNA, actin, tubulin and myosin and with circulating proteins such as thyroglobulin.[16] Furthermore, the number of precursor B cells to murine thyroglobulin in normal mice (about 1 per 4000 lipopolysaccharide (LPS)-responsive B-cell precursors) is comparable to the number of precursors to

some foreign antigens.[17] However, autoantibodies have not so far been reported to the alloantigens of the major blood group system or to MHC antigens.

What seems generally accepted now is that clonal elimination in the T helper cell compartment is the main mechanism involved in maintaining self-tolerance, but that this may principally involve cells with high-affinity receptors for self antigens. However, precursor T cells of lower affinity, but capable of being stimulated by a variety of normal host proteins, seem to occur naturally. It has been proposed that it is the activation of these T cells with a concomitant increase in the affinity of their receptors, not the presence of autoantibodies *per se*, which initiates a disease process.[18]

Advances in our understanding of immune processes

In the 12 years which have elapsed since the earlier report,[1] there have been major advances in our understanding of how the immune system works and in our ability to manipulate immune responses. For both the human and the murine systems particularly, much more is now known about subsets of T and bone marrow-derived (B) cells, markers for these cells, of quantitating B and T cell memory, and of the lymphokines involved in the interaction between cells. A major recent finding was the classification of T helper cells into two groups which had distinct patterns of lymphokine secretion so that the prospect of being able to induce or suppress the activation of different T effector and B cells may be realized in the near future. Side by side with this increased knowledge are advances in the development of both screening and assay methodologies for antigens and antibodies.[19]

A re-evaluation of testing the efficacy and safety of anti-self vaccines

In the light of these considerations and developments, it was decided that now was an appropriate time to carry out another review of the processes involved in the evaluation of the efficacy and safety of antifertility vaccines. The need to achieve a very high level of safety would focus attention on both immunological and endocrine aspects. Vaccines were developed as a prophylactic measure to prevent disease caused by infectious agents. Provided their use (especially in humans) caused only low levels of morbidity and especially mortality, they were readily accepted by the medical profession and by the general public, who viewed the low incidence of side reactions as much more acceptable than the ravages and sequelae of the natural infection.

Antifertility vaccines, unlike other vaccines, are given to healthy people to prevent a normal, non-disease-inducing physiological process. If pregnancy does occur after vaccination, the ongoing immune response may constitute a risk to the developing fetus. Furthermore, there is a variety of alternative

methodologies for the same purpose. To minimize risks and to ensure that such vaccines are an acceptable addition to the range of fertility-regulating methods already available, the Task Force has re-asserted the general principle that target antigens should be present transiently and in low amounts relative to a predicted antibody response and should be restricted to gametes and/or the early products of fertilization. Other projects aimed at developing antifertility vaccines have less restricted approaches in which the immune response to the target antigen cross-reacts with other hormones[20,21] or in which a hormone which is continually present is the target antigen.[22]

The current schedules for the safety testing of vaccines in many countries before general release of the product should detect serious untoward effects which occur at relatively high frequencies, e.g. 1 in 10000 cases. Serious side-effects which occur at substantially lower frequencies are not detected until a vaccine is administered to millions of people. In such cases, e.g. the occurrence of cases of Guillain–Barré syndrome after the administration of the swine influenza vaccine in 1976 in the USA, or of severe neurological disturbances following immunization with the whole cell pertussis vaccine, it has been extraordinarily difficult to establish a cause-and-effect relationship between the vaccine's administration and the associated complaint.[23] Similarly, it may not be possible to prevent such rare events occurring after administration of an antifertility vaccine. However, if modern endocrinological or immunological assays are considered good predictors of these effects, such assays should be used.

Vaccine efficacy has been measured in several ways, but mainly in terms of disease prevention. Two indices – cost-effectiveness, judged by cost–benefit analysis, and cost-saving, judged by a saving in nett medical care – have been used[24] but are only applicable after large-scale vaccine administration. In the development of vaccines, seroconversion has been the major test used as an index of efficacy and is currently being applied as a test for the efficacy of hCG vaccines. There is increasing evidence that the measurement of cell-mediated immune responses may also be important in assessing the efficacy of some vaccines, suggesting that appropriate assays for these reactions need to be established.

Specific objectives

The specific objective of this symposium is to review aspects of present and past work on the development of antifertility vaccines which are particularly relevant to the testing of their efficacy and safety, with special attention to immunological and endocrine aspects. It is anticipated that recommendations will evolve which might serve not only as guidelines for future work on the development of vaccines against self antigens, possibly including antitumour vaccines, but will also be useful points to consider by those developing antidisease vaccines.

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2 The process of reproduction in humans: antigens for vaccine development

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Introduction

The aim of this chapter is to review briefly the essential steps in the process of reproduction in humans. This is an exceedingly complex topic with many facets which are of particular interest to both endocrinologists and immunologists. The sequence of events which occur in both the female and the male will be discussed and antigens described which could be considered, in an initial screening, to qualify as candidates for vaccine development. Points for and against antigens in each category will be made with the aim of arriving at a short list for serious consideration. It must be stressed, however, that this list of candidate antigens is not exhaustive but serves only to provide examples of some of the options currently available for vaccine development.

An outline of the reproductive process

The sequence of events involved in reproduction in both the male and female and which result in implantation of the blastocyst in the uterus are outlined in Figure 2.1. They can be conveniently divided into three stages. The first is the production of the male and female gametes.

Production of gametes

In both sexes, the hormonal background involves the secretion from the hypothalamus of gonadotrophin-releasing hormone (GnRH), sometimes called luteinizing hormone-releasing hormone (LHRH). This is carried in the blood to the pituitary where it binds to membrane receptors on pituitary gonadotroph cells to induce the synthesis and release of two hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

In the male

FSH binds to receptors on Sertoli cells in the testes, and thereby influences the metabolic and secretory activities of this cell which are believed to be fundamental to the maintenance of the germ cells. Among the secretory proteins is a specific androgen-binding protein (ABP) which assists in the

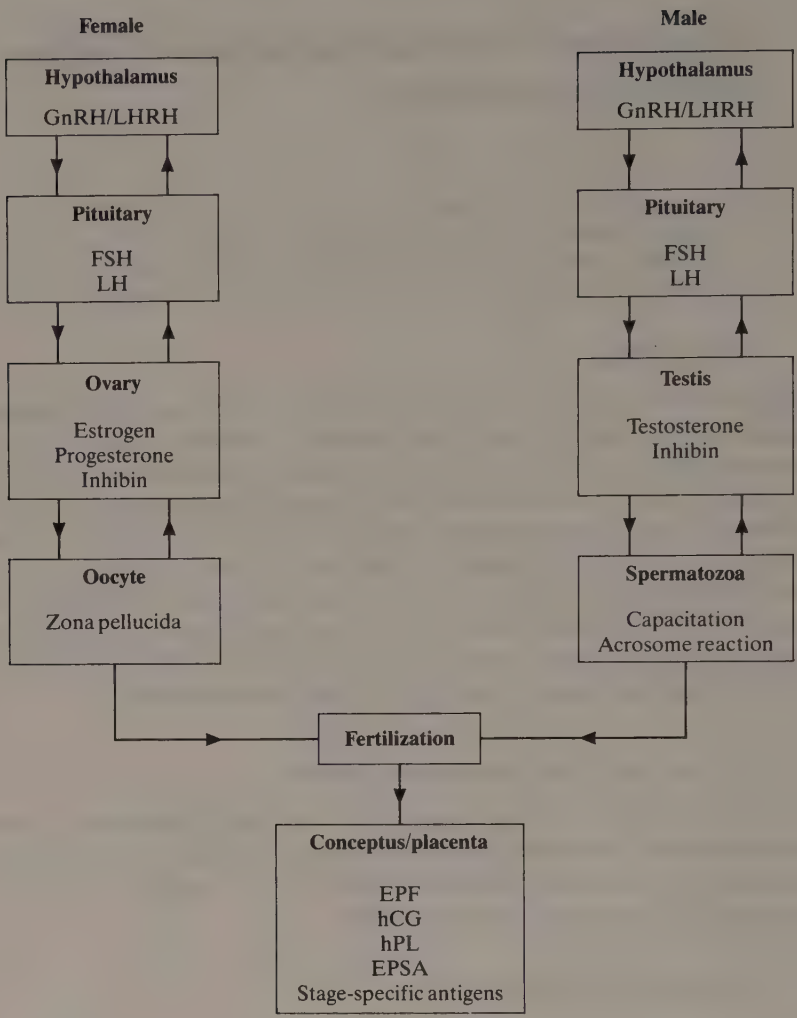

Fig. 2.1 Early events in human reproduction. (GnRH, gonadotrophin-releasing hormone; LHRH, luteinizing hormone-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; EPF, early pregnancy factor; hCG, human chorionic gonadotrophin; hPL, human placental lactogen; EPSA, early pregnancy-specific antigen.)

transport of testosterone within the germinal epithelium, so facilitating the interaction of the androgens with receptors on the germ-cell membrane. The androgen–receptor complexes are endocytosed into the germ cells.

LH reacts with receptors on the membrane of Leydig cells in the testes and thus activates the biosynthesis of testosterone, which is also necessary for

spermatogenesis. Two hormones formed in the testis, testosterone and inhibin secreted by the Sertoli cell, exercise a feedback regulatory function on FSH and LH production.

There is a blood–testis barrier which is thought to have a number of functions, such as the maintenance of a favourable spermatogenic environment and protection of the developing germ cells from environmental toxins, mutagens and sperm-specific autoantibodies.

The spermatozoa leave the testis and pass through the epididymis where they mature and acquire reproductive competence, i.e. they become fertile. This involves *inter alia* development of the acrosome structure and acquisition of motility. The epithelial cells in the epididymis also secrete a variety of proteins, some of which become associated with the sperm. A proportion of these possibly integrate with the sperm plasma membrane (as they are not easily dislodged by washing) and influence the various features of sperm maturation.

Male animals, immunized with homologous sperm or testicular antigens in Freund's complete adjuvant, make an immune response against these materials.[1] In some species, the blood–testis barrier is broken and the animals develop experimental allergic orchitis which leads to aspermatogenesis. Both antibody and effector T cells may be involved in the development of the lesion.

In the female

Follicles in various stages of growth, interstitial cells and connective tissue form the bulk of the ovary. Follicles generally consist of a basement membrane, granulosa cells and usually one oocyte and are surrounded by interstitial cells. FSH binds exclusively to granulosa cells in the growing follicles. It stimulates the formation of receptors for itself and for LH and enhances ovarian steroidogenesis, including the secretion of progesterone. Progesterone, and oestrogens secreted from the interstitial cells, form a feedback control loop which regulates the secretion of FSH and LH.

Receptors for LH are present in the granulosa, the corpora lutea and on the cells on the basement membrane (theca interna). Their number increases greatly during follicular development. Oestrogen also enhances follicular growth, ovulation and luteinization by increasing the sensitivity of the follicle to the gonadotrophins. At mid-cycle, oestrogens released from large follicles trigger a surge of LH production which induces ovulation, that is, the rupture of the follicle and release of the oocyte with its acellular coat, the zona pellucida, and some attached granulosa cells.

The corpus luteum forms after ovulation from the remaining granulosa cells and continues to produce progesterone for 14 days. It then regresses (in an infertile cycle) or is rescued by chorionic gonadotrophin (CG) from the conceptus in a fertile cycle.

Autoantibodies to a variety of proteins, especially those of the zona pellucida, have been shown to be present in different women. In experimental

animals, injection of antibodies specific for these proteins can prevent conception.[2,3,4]

Events leading to fertilization

Introduction of the sperm into the female reproductive tract may lead to fertilization of the oocyte. The sperm undergo physiological changes in the female reproductive tract before they are able to penetrate the oocyte. The first stage is capacitation and involves subtle changes in the sperm membrane and takes place over several hours. Capacitation triggers the events leading to the acrosome reaction. The acrosome is a sac containing several enzymes whose hydrolytic action allows penetration of the annulus oophorus and the zona pellucida. The acrosome reaction involves fusion between the plasma membrane of the sperm and the outer acrosomal membrane, resulting in the release or activation of the enzymes in the acrosome. In viable sperm, the acrosome reaction occurs in the oviduct, at or about the time when the sperm reaches cells surrounding the zona pellucida. The precise trigger for this reaction is not known, but the reaction itself is essentially autolytic and takes place in part in dying sperm which have not reached the oviduct. Specific chemical groups appear to be involved in the attachment of the sperm to the zona pellucida as sperm will not bind to eggs that are already fertilized. The entire process is highly species specific.

Post-fertilization events

After fertilization, the embryo begins the cleavage divisions as it moves through the oviduct. Several divisions have occurred by the time the embryo reaches the uterus. Growth continues with the formation of a blastocyst, at which stage implantation in the wall of the uterus takes place, between days 6 and 13 after fertilization.

The blastocyst consists of the developing embryo (inner cell mass) which is eccentrically placed within a single layer of trophoblast cells which encloses the blastocoelic cavity. The trophoblast develops into the extra-embryonic part of the placenta.

The production of CG by the trophectoderm of the embryo can be detected 6–9 days after conception. The major function of CG is to 'rescue' the corpus luteum whose production of progesterone maintains the uterus in a receptive state for implantation and the establishment of pregnancy.

Candidate antigens for fertility-regulating vaccine development

There are thus two classes of potential target antigens:

1. hormones required for the continuing generation of the gametes and

the control of their production, as well as hormones secreted by the early conceptus;

2. cell-associated proteins, many present as glycoproteins, which may be expressed at or be present on the surface of the gametes or the early conceptus, or exposed during fertilization.

The general criteria on which these antigens can be selected as candidates for antifertility vaccine development and their relative advantages and disadvantages for this purpose are summarized in Table 2.1.

Hormones as candidate antigens

Hypothalamic hormones

Immunization against GnRH can produce an indirect antifertility effect by reducing the levels of gonadotrophin secretion. This leads to ovarian and uterine atrophy in the female and testicular atrophy, spermatogenic arrest and involution of the prostrate and seminal vesicles in the male. In addition, the consequent reduction in steroid hormone secretion leads to a decline in secondary sexual characteristics and libido. These effects have been demonstrated in a number of animal species, including at least two infra-human primates.[5-9] The use of an anti-GnRH vaccine would require testosterone supplementation to overcome many of these side-effects and would run the risk of inducing immunopathology in the pituitary – considerations that detract from the utility of the approach.

Pituitary hormones

Wide species differences have been observed in the physiological responses to anti-FSH immunization.[10,11,12] Varying degrees of spermatogenic arrest in males and inhibition of follicular growth in females have been described in a number of studies carried out in different infra-human primate species.[13,14] The secretion of pituitary LH and gonadal steroidogenesis are not affected by this treatment and there appears to be no significant reduction in the libido of the treated animals. No adverse physiological or behavioural effects have been observed in bonnet monkeys actively immunized with ovine FSH for periods of up to 13 years.[15]

Although the long-term immunopathological sequelae of immunization with homologous FSH have yet to be assessed and the approach itself is not free of controversy,[16] the encouraging data from monkeys chronically immunized with ovine FSH suggest that further work in this area may be profitable.

The development of an anti-LH vaccine, however, has been considered by some to be less attractive because of possible endocrine disturbances and their sequelae and the potential for inducing autoimmune disease in the pituitary. However, in a number of studies carried out in several animal species,

Table 2.1. *Criteria for fertility-regulating vaccine development*

Advantages of fertility-regulating vaccines
(a) Long duration of effect from single injection or course of immunization
(b) Absence of pharmacological activity
(c) Absence of user-failure risk
(d) Administration by method with high level of acceptability
(e) Low cost

General requirements for antigen selection
(a) Secreted or membrane-bound molecules (preferably protein) of structural/functional necessity for gamete maturation and/or interaction
(b) Secreted by or found only on intended target cells and not in other tissues
(c) Immunological inhibition or removal results in antifertility effect
(d) Effective immune response is free of unacceptable side-effects
(e) Molecules conserved across species so that meaningful preclinical safety and efficacy studies can be conducted in appropriate animal models

Vaccine candidates

(a) *Sperm membrane antigens*

Effective:	prefertilization
If used by females:	molecules should be restricted to sperm in upper segment of genital tract
If used by males:	molecules must be expressed/exposed only in post-testicular phases of sperm maturation
Advantages:	in females – target material is immunologically foreign and contained within lumen of genital tract
Disadvantages:	in females – adequate level of immunity needed in lumen of genital tract to combine with high antigen load
	in males – risk of auto-immune reactions in testis and male genital tract

(b) *Ovum (zona pellucida) antigens*

Effective:	prefertilization
If used by females:	molecules must be expressed/exposed only in peri/post-ovulatory oocytes
If used by males:	not applicable
Advantages:	small amount of target antigen restricted to peri-ovulatory follicles and to lumen of genital tract for limited period of time
Disadvantages:	in females – risk of auto-immune reactions in ovary
	in males – not applicable

(c) *Trophoblast membrane antigens and products*

Effective:	postfertilization
If used by females:	molecules must be exposed on or secreted by peri-implantation trophectoderm
If used by males:	not applicable
Advantages:	target molecules only present when fertilization has occurred
	target molecules accessible to secretory immunity in lumen of genital tract and/or come into contact with blood of vaccine recipient at early stage of implantation

including infra-human primates,[17,18] immunization against LH has been shown both to suppress ovulation and to disrupt implantation, either by induction of a defective corpus luteum or by removal of luteotrophic support as a result of the anti-LH antibodies cross-reacting with hCG. Immunization of infra-human primates with the intact beta subunit of hCG, which results in the production of antibodies which cross-react with LH, has shown no evidence of autoimmune pathology. Other studies, in which rhesus monkeys have been chronically immunized with ovine beta-LH, have also failed to demonstrate any overt toxic effects.[19]

Gonadal hormones

Antibodies raised to conjugated testosterone and to estradiol and progesterone can disrupt fertility in the male and female respectively.[20,21] In the male, this treatment results in varying degrees of inhibition of spermatogenesis, regression of the accessory glands and reduction of libido. In the female, follicular development is abnormal, ovulation is impaired and menstrual cycles become irregular. The variations in the nature and magnitude of these effects probably reflect a compensatory increase in the production of pituitary gonadotrophins. These undesirable side-effects would make vaccines directed at these target hormones unattractive and probably unacceptable for clinical use.

Hormones of the conceptus

The principal hormonal products of the conceptus are placental lactogen (PL) and CG. Passive[22,23,24] and active[25,26] immunization of pregnant rodents, rabbits, squirrel monkeys and baboons with partially purified hPL resulted in a substantial level of pregnancy interruption. However, when baboons were immunized with highly purified haptenated homologous bPL, no antifertility effect was observed, although high levels of anti-bPL antibodies were present. This suggests that any effect of the available antibodies was more than compensated for by the rapidly increasing production of the hormone,[27] that the antifertility effect may have been cell mediated rather than antibody mediated,[28] or that the antifertility effect resulted from immunity to placental or fetal contaminants in the impure hPL preparations.[29]

In contrast, a pronounced antifertility effect has been achieved in a number of studies carried out in several infra-human primate species using vaccines based on either the complete beta subunit of hCG[30,31,32] or a synthetic peptide representing the C-terminal 37 amino acid sequence of the beta subunit of the hormone, conjugated to a diphtheria toxoid carrier.[33–36] Phase I clinical trials of vaccines incorporating the whole beta subunit of hCG conjugated to tetanus toxoid[37] and the C-terminal 37 amino acid peptide of beta hCG conjugated to diphtheria toxoid,[38] have demonstrated the ability of these preparations to elicit anti-hCG antibodies in the absence of serious side-effects.

It should be noted that the alpha subunits of some of the pituitary and conceptus hormones – CG, LH, FSH and the thyroid-stimulating hormone (TSH) – are identical, thus preventing the use of intact LH, CG or FSH in a vaccine.

Cell-associated proteins as candidate antigens

Sperm-associated antigens

Antisperm or anti-ovum vaccines which are effective at the pre-fertilization stages of the reproductive process are likely to find wide acceptance. The development of antigamete vaccines, therefore, represents an attractive line of research.

Studies have been carried out since the very early days of modern immunology to induce antisperm immunity in humans. Earlier studies involved the use of whole semen or testis extracts, whereas more recent studies have used natural or synthetic fragments of sperm antigens or enzymes.

The usual objection to the use of an antisperm vaccine in men, the production of autoimmune orchitis, may be overcome by developing a vaccine based on antigens which are expressed only on post-testicular sperm, perhaps expressed or acquired in the epididymis.

Cell-surface antigens are first expressed in spermatogenesis in mice, rats and rabbits, at the pachytene spermatocyte stage. This stage occurs during premeiosis and when the spermatocytes are inside the blood–testis barrier. After meiosis and the formation of the haploid spermatids, other antigenic determinants are expressed. The flagellated spermatozoa released into the lumen of the seminiferous tubule are carried in a fluid of unique composition into the duct of the epididymis. The fluid is rich in potassium and also contains a number of proteins.

Studies with human epididymal tubules in organ culture have shown that androgens induce the secretion of five proteins (molecular weights 21 000–69 000), two of which have the same electrophoretic mobility on gels as two proteins obtained from washed ejaculated sperm.[39] Such membrane proteins appear to be acquired in the epididymis and subsequently become bound to the membrane of the acrosome. It is inferred that they are linked to post-ejaculatory characteristics crucial to fertilization, such as capacitation, the acrosome reaction, penetration of the cumulus oophorus, and zona pellucida binding. These proteins are thus potential targets for immunological intervention.

Much of the research carried out in the antisperm vaccine area has been conducted on the basis that an antisperm vaccine would be used in women. This would have the advantages that the vaccine would contain foreign material, likely to be highly immunogenic and free of cross-reactivity, and the resulting immune response would be directed at a target which was only present at coitus and was sequestered in the lumen of the female genital tract. On the other hand, there are two potential problems: one is the large amount

of antigen to be 'neutralized' at each coitus, and the second is the requirement for an adequate level of antibody or immune cells to be secreted into the lumen of the upper regions of the female genital tract. An attractive approach to overcoming the first problem is to select for vaccine development antigens that are only expressed or are 'available' on sperm when they reach the upper segment of the oviduct where fertilization usually occurs. Such antigens are likely to be relevant to the processes of sperm motility and fertilization and, indeed, sperm subpopulations expressing such antigens have been described.

Sperm antigens in this category include the enzymes acrosin, hyaluronidase and sperm-specific isoenzyme of lactic dehydrogenase (LDH-C_4). By virtue of their known roles in the fertilization process, the former two enzymes are attractive candidates. However, active isoimmunization of sheep with either or both of these enzymes failed to induce a contraceptive effect,[40] despite in-vitro experiments which showed a profound effect of antibodies to these enzymes on enzyme activity, sperm attachment and penetration rates. In contrast, a significant reduction in fertility has been achieved in female baboons immunized with a prototype vaccine incorporating a 15 amino acid peptide of LDH-C_4,[41] suggesting that the problems of antigen load and accessibility by components of the immune system can be overcome. It is not known whether sperm in a particular region of the reproductive tract were more susceptible to or were preferentially attacked by the immune system components.

Ovum-associated antigens

Most studies aimed at the development of a vaccine against the ovum have involved immunization with the zona pellucida (ZP). Pronounced antifertility effects have been observed in several animal species immunized with solubilized whole ZP or with ZP-derived fractions.[42,43] Marmosets and baboons immunized with solubilized whole porcine ZP exhibited a marked reduction in fertility but this was accompanied, in many cases, by disturbances in ovarian structure and function, as manifested by menstrual cycle disruption and prolonged periods of amenorrhea.[44,45] Attempts are being made to isolate and characterize individual ZP proteins; one candidate is a protein with a M_r of 5.5 kDa whose biological function is not yet known but which has been used to immunize female squirrel monkeys.[46] Although some evidence of an antifertility effect was observed in these studies, this was accompanied by abnormal ovarian histology and endocrinology. To avoid some intra-ovarian structural or functional changes, it has been suggested that only ZP antigens expressed in the late stages of oogenesis or post-ovulation should be candidates for vaccine development.

Conceptus-associated antigens

The potential non-hormonal targets in the conceptus are either embryo-specific products which act as 'pregnancy signals' to the mother, or tissue-specific membrane-associated antigens in the early trophoblast. Two have

been described: an early pregnancy factor (EPF) which appears to have an immunosuppressive activity,[47] and stage-specific antigens expressed on the surface of the early placenta.[48] A vaccine directed against EPF might function by de-suppressing the 'natural' immune response to the embryo.

The early trophoblast, which duly develops into part of the placenta, should also be a source of many candidate antigens as they might be restricted to the very early stage of development. Studies of these antigens may only be relevant if carried out in infra-human primates; recent advances in techniques of in-vitro culture of embryos beyond the implantation stage of development are important to such studies.

Placental antigens

The direct contact between the maternal blood supply and the placenta makes the early placenta an easily accessible target for the maternal immune system. A number of placenta-specific proteins has been described and isolated in a pure form. Passive immunization of pregnant cynomolgus monkeys with the IgG of rabbit antisera raised to one of these, human SP-1, led to a high number of abortions.[49] Similar results were obtained when cynomolgus monkeys were actively immunized with human SP-1, but active immunization of rhesus monkeys and baboons with rhesus SP-1 failed to produce a significant antifertility effect, even though high levels of anti-SP-1 antibodies could be detected in the treated animals.[50]

Part of the difficulty with this and some of the other approaches involving cell-associated proteins is the lack of availability of sufficient material for adequate molecular studies and for biological testing. The newer approaches of establishing gene banks of different cell types and the recently developed techniques of screening for plasma membrane associated antigens should greatly facilitate the identification and production of putative antigens as vaccine candidates. When combined with the steadily increasing availability of monoclonal antibodies, the task of identifying, making sufficient amounts of, studying in detail and testing putative antigens will become much easier. A recently initiated international, multicentre study involving the use of these new technologies has already produced encouraging data.[51]

General considerations

As was stressed previously, a vaccine to control human fertility must be safe (free from any pathological sequelae), effective (with an acceptably low level of Ir gene effects, and independent of age etc.) and acceptable (free from side-effects). Care is needed to make sure that any immunization schedule is completely effective as a contraceptive; in particular, an immunization schedule which is only partly successful may run the risk of inducing fetal damage or incorrect development. This concern may be most relevant in the case of a vaccine directed to a product(s) of the gametes or the conceptus.

Another aspect is the need for reversibility, which means that the continuing

or pulsed appearance of the target antigen in the normal, immunized recipient should not act as a stimulus to an ongoing immune response, induced in the first place by vaccination. This favours the use of a target antigen which *per se* is poorly endowed with T cell epitopes. Of the different sources of cell-associated antigens mentioned above, the generally low expression of MHC antigens on the gametes in particular favours their exploitation as a source of candidate antigens for vaccine development. This aspect also implies that the duration of immunity required for vaccination to be regarded as successful is dependent exclusively on the immunization protocol.

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3 Animal models for assessing the safety and efficacy of antifertility vaccines

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Introduction

The principal objective of this chapter is to review the factors to be taken into consideration when selecting the animal species in which the preclinical safety and efficacy evaluations of prototype antifertility vaccines are to be carried out.

The specific points to be addressed include: the relevance of the animal models in relation to the vaccine to be tested; the availability of the animals; the feasibility of carrying out all of the required studies in the animal models selected; examples of the factors to be taken into account for different antifertility vaccines; and the animal studies that need to be carried out prior to Phase I, Phase II and Phase III clinical trials of these preparations.

Relevance of the animal model

Relevance is probably the most important question to be addressed and probably also the most difficult to answer in that so many factors need to be taken into consideration, depending on the particular vaccine to be studied, the objectives of the study to be conducted, and the parameters to be measured. These factors include the similarities of the animal models to the human in terms of their reproductive biology and immunological responsiveness (both quantitative and qualitative), and in terms of the antifertility mechanism and immunopathological reactions and sequelae associated with the use of the vaccine.

Other important factors to be taken into consideration include: what is known about the various animal models in terms of their reproductive performance; the natural incidence of pathology and fetal abnormalities; their susceptibility to disease; and known reactions to experimentation involving the administration of drugs or the injection of vaccines.[1]

Availability of the animal model

Having selected animal species on the basis of their relevance, the next question to be addressed concerns the availability of the animals in sufficient

numbers to carry out the proposed studies in a way that will permit the generation of statistically valid data. This is not a problem if the usual laboratory rodents are to be used, but can present a major difficulty if more exotic species are required, such as the higher primates.

Feasibility of use of the animal model

Finally, it is pointless to select animal models whose use presents logistical, legal or financial limitations to the studies than can be carried out with them. For example: can the vaccine be administered by the proposed route and at the required frequency and dose? Can body fluids and tissues be taken in the quantities required for the laboratory examinations to be carried out during the in-life phase of the studies? Can the animals be autopsied during and at the end of the study for post-mortem examinations and analysis? The last point takes on particular significance with regard to the protected status of some of the higher primate species, particularly the apes.

Examples of animal models used in ongoing vaccine development

To determine how these diverse and conflicting requirements of relevance, availability and feasibility can be reconciled with the specific requirements for fertility-regulating vaccines directed at different targets, it may be informative to consider them in the context of the development of three antifertility vaccines directed at different and distinct reproduction-specific targets.

Antisperm vaccines

It is envisaged that antisperm vaccines will be used, at least initially, by women. Being derived from a 'foreign' cell, they would have the dual advantages of increased immunogenicity and reduced risk of inducing autoimmunity. However, studies would need to be carried out to determine if the immune response elicited by a vaccine claimed to be sperm specific, did, in fact, lead to cross-reactions with other tissues in the immunized female and, if cross-reactivities were observed, to define these in terms of their nature, location and potential pathological sequelae.

A much greater potential for inducing autoimmunity and its consequences would be involved with the use of antisperm vaccines by men. In this situation, care would need to be taken to assess the risks of the vaccine producing subtle changes in the testicular germinal epithelium – which could result in the production of abnormal spermatozoa which may only be detected as a carry-over effect to the embryo – as well as more obvious pathology such as generalized autoimmune orchitis. Preclinical safety studies would need to be carried out to look for evidence of these subtle and gross events and would

Table 3.1. *Interspecies comparison: female*

Species	Cycles	Cycle length (days)	Uterus	Implantation	Placental membrane	Gestation length (days)	Litter size
Mouse	E	4–5	Bicornuate	Interstitial	Hemotrichorial	19–21	10–12
Rat	E	4–5	Bicornuate	Interstitial	Hemotrichorial	21–23	6–12
Guinea pig	E	15–17	Bicornuate	Interstitial	Hemomonochorial	59–72	2–5
Hamster	E	4	Duplex	Interstitial	Hemotrichorial	15–16	5–9
Rabbit	E	IO	Duplex	Superficial	Hemodichorial	29–35	4–10
Dog	E	60–64	Bicornuate	Superficial	Endotheliochorial	60–63	4–8
Cat	E	IO	Bicornuate	Superficial	Endotheliochorial	56–63	3–5
Pig	E	21	Bicornuate	Superficial	Epitheliochorial	112–115	6–12
Cow	E	21	Bicornuate	Superficial	Syndesmochorial	277–290	1–2
Sheep	E	16.5	Bicornuate	Superficial	Syndesmochorial	144–152	1–3
Goat	E	20–21	Bicornuate	Superficial	Syndesmochorial	146–151	1–3
Marmoset	E	27–29	Simplex	Superficial	Hemomonochorial	141–146	2–3
Squirrel monkey	E	8–10	Simplex	Superficial	Hemomonochorial	150–170	1
Macaque	M	24–32	Simplex	Superficial	Hemomonochorial	160–170	1
Baboon	M	32–36	Simplex	Superficial	Hemomonochorial	175–180	1
Chimp	M	34–35	Simplex	Interstitial	Hemomonochorial	216–260	1
Human	M	28–30	Simplex	Interstitial	Hemomonochorial	36–40 wk	1–2

Note:
E = estrus; M = menstrual; IO = induced ovulator.

involve functional and structural evaluations of the testes of immunized animals as well as an assessment of their reproductive behaviour and the normality of their offspring. Again, studies would need to be carried out to determine if the immunization of males with an antisperm vaccine produced cross-reactive immunity against non-reproductive tissues.

The animal models in which these studies are conducted depend primarily on the particular sperm antigen used in the vaccine. For example, if this antigen is common to the spermatozoa of different animal species and appears to be restricted to the same location on the sperm cell, it is reasonable to assume that it has the same function in all cases and that any one of these animals would represent a suitable model. In this case, other factors such as the degree of similarity of the reproductive anatomy/physiology and of the immune system of the animal and of humans would come into play in making the choice of the most relevant model.

For an antisperm vaccine to be effective, either in women or men, antibodies and/or immune cells must be able to gain access to the lumen of either reproductive tract in sufficient quantities to 'neutralize' the large numbers of spermatozoa that are deposited at coitus and that are constantly being produced in the fertile adult male. Although the anatomical aspects of intercourse are essentially the same in most mammalian species, the site of semen deposition is quite different (Tables 3.1 and 3.2). For example, rodents

Table 3.2. *Interspecies comparison: male*

Species	Spermatogenesis (days)	Ejaculate volume (ml)	Sperm concentration ($\times 10^{-6}$/ml)	Ejaculate characteristics	Semen deposition	Accessory glands			
						Ampulla	SV	Prostate	BUG
Mouse	34–35	0.1±	50	Vaginal plug	Uterine	±	+	+	+
Rat	48–53	0.1	58	Vaginal plug	Uterine	+	+	+	+
Guinea pig		0.4–0.8	80–100	Vaginal plug	Uterine	−	+	+	+
Hamster	35–36	0.15±	80	Vaginal plug	Uterine	+	+	+	+
Rabbit	48–51	0.4–6.0	60–200	Incipient plug	Vaginal	±	±	+	+
Dog	42	7–10	60–300	Very dilute	Uterine	±	−	+	−
Cat		0.01–0.50	50–60	Highly viscous	Vaginal	−	−	+	+
Pig	34–35	5.0	5000	Voluminous	Vaginal	−	+	+	+
Bull	54	4–5	4000–5000	Little accessory fluid/high concentration	Vaginal	+	+	+	+
Sheep	49	0.7–2.0	2000–5000	Little accessory fluid/high concentration	Vaginal	+	+	+	+
Goat		0.2–2.5	1000–5000		Vaginal	+	+	+	+
Squirrel monkey	39	0.2–1.5	80.8–310.9	Coagulation	Vaginal	−	+	+	+
Macaque	36	0.6–4.5	100.2–830.1	Instant coagulation	Vaginal	±	+	+	+
Baboon	42			Coagulation	Vaginal	+	+	+	+
Chimp				Mostly coagulation upon ejaculation	Vaginal	+	+	+	+
Human	64	2–6	50–150	Slight coagulation	Vaginal	+	+	+	+

Note:
SV = seminal vesicles; BUG = bulbourethral glands.

and dogs deposit semen directly into the uterus, whereas in all primates studied to date, including humans, semen is deposited in the vagina. In primates, the cervix is a major barrier to sperm ascending the reproductive tract, and immunoglobulins in the cervical mucus have a major impact on sperm survival and transport. Thus, the model species should have intravaginal insemination and a cervical barrier to sperm transport. Similarities to the human situation in terms of antigen presence, location and concentration, the site and nature of gamete interaction and the composition of the genital tract secretions, are also desirable.

Anti-ovum vaccines

As an anti-ovum vaccine could only be used by women, the same concerns would apply to the evaluation of this vaccine as were described for the use of an antisperm vaccine in men (see preceding section).

Molecules within the ovum and its plasma membrane are not considered attractive as antifertility immunogens, either because of their lack of tissue specificity or because of the potential for inducing sublethal damage to the ovum which may result in teratology. The emphasis for the development of an anti-ovum vaccine, therefore, is concentrated on the zona pellucida (ZP), the acellular glycoprotein shell which forms around the oocyte during the later stages of oogenesis and which plays an important regulatory role in the process of sperm–ovum interactions involved in fertilization.

Although the antifertility efficacy of immunization with ZP has been demonstrated in several animal models by a number of investigators (see Chapter 2), detailed evaluations of the safety of this approach have not been carried out. Such studies will need to include routine tests for cross-reaction with other body constituents, with particular attention being given to those tissues producing and/or secreting other glycoprotein molecules. In addition, a detailed analysis would need to be carried out to determine if anti-ZP immunization was associated with deleterious changes in the oocyte and in the granulosa cells of the ovarian follicle, both of which are believed to contribute components of the ZP.

A careful analysis of the molecules comprising the ZP will need to be made in order to identify and select acceptable candidate immunogens for vaccine development. Once such a vaccine has been developed, meaningful safety and efficacy studies can be carried out. The selection of the animal species in which these tests are to be conducted will again be based primarily on the similarities of antigen presence, location and concentration, the nature of gamete interaction and the composition of the genital tract secretions of the various models compared to the corresponding human situation. Species with obviously different ZP to the human, such as the rabbit, may not be appropriate candidates.

Anti-hCG vaccines

A fundamental difference between an anti-human chorionic gonadotrophin (hCG) vaccine and vaccines directed against sperm and the ZP is that the chorionic gonadotrophins are essentially primate specific and, furthermore, form a family of related but not identical molecules within the primate order. In addition, the degree of chemical and immunological similarity between the biologically important components of the chorionic gonadotrophins of different primate species and their closest known molecular relatives, the pituitary luteinizing hormones (LH), decreases as the phylogenetic scale is descended.

These factors necessitate that the majority of the preclinical safety and efficacy studies with an anti-hCG vaccine need to be carried out in a non-human primate as close as possible, phylogenetically, to the human. In this regard, the gorilla, chimpanzee and orangutan are the most appropriate primate species but their protected status prevents their use for studies, such as this, which require both intensive in-life evaluations as well as sacrifice and post-mortem examinations. For this reason, the baboon was selected as the most relevant animal model for evaluating the safety and efficacy of the anti-hCG vaccine developed by the WHO Task Force on Vaccines for Fertility Regulation.

However, antibodies raised to the β-subunit of hCG (β-hCG) cross-react with hCG, human LH (hLH) and baboon CG (bCG) but not with baboon LH (bLH). Therefore, evaluation of the safety of heterologous immunization with vaccines based on β-hCG or its derivatives, in baboons or any lower primate species, is unlikely to yield meaningful data in terms of the direct or indirect interference with hLH production and/or function that might occur when a β-hCG vaccine is used in the homologous human situation. Therefore, the development of an animal model system in which immunization is carried out with a vaccine based on the corresponding antigen of the homologous molecule in the selected animal, should be developed for comparison with its human counterpart.

Safety studies

The studies identified by WHO[2,3] and by national regulatory authorities[4] for the preclinical reproductive toxicity/teratogenicity testing of new drugs are designed to identify drug-associated adverse effects at three susceptible stages of reproduction – gametogenesis, fetal organogenesis, and peri-/post-natal development.[1] In general, the same studies could be carried out with antifertility vaccines, although some, such as the effect on gametogenesis in the male, may not be relevant for vaccines directed against oocyte, placental and other female-specific antigens, and vice versa. In view of their novelty, the amount of relevant experience with fertility-regulating vaccines is limited and, consequently, the regulatory requirements for the preclinical toxicological

testing of such vaccines are not clearly defined. The following information is based, therefore, on the studies conducted and/or planned for the anti-hCG vaccine developed with Task Force support and is provided as an example only.

Prior to the Task Force's studies with an anti-hCG vaccine, there was little, if any, relevant previous experience nor regulatory requirements to indicate the type of preclinical toxicity and safety studies that should be conducted with an antifertility vaccine. The pre-Phase I anti-hCG vaccine toxicology, immunosafety and efficacy studies carried out by the Task Force,[5] therefore, were based largely on those used for assessment of new drugs, irrespective of composition, mode of action and intended use. These studies involved assessments of acute, subacute and chronic toxicities of the complete vaccine formulation, as well as various combinations of its individual constituents, and were carried out at several dose levels in mice, rats, rabbits and baboons.

Prior to Phase I clinical trials

At this stage of vaccine development, the objective is to assess if the vaccine or its constituents produce toxic or other unacceptable adverse side-effects. The studies include, therefore, general tests for chemical toxicity, similar to those carried out for conventional drugs, and specific tests for immunotoxicity of the vaccine. Although efficacy will not be tested in the Phase I clinical trials, which are usually carried out in previously electively sterilized volunteers, the ability of the vaccine to regulate fertility in an appropriate animal model needs to be demonstrated at this stage (see later). The pre-Phase I toxicity studies can be broadly divided into two types.

(a) *Chemical toxicity* of the vaccine dosage form, of its individual components and of its breakdown/metabolic products.

The purpose of chemical toxicity studies is to determine if the complete vaccine formulation and its individual components will be free of undesirable or unacceptable toxic side-effects at the dose proposed for clinical use. The studies include assessments of acute and subacute toxicity in mice and rats and intramuscular tolerance studies in rabbits. Typically, acute toxicity studies are restricted to body weight measurements and daily observations for detection of gross and overt changes in general health status, whereas subacute toxicity studies include in-life haematological, biochemical and urine analyses and post-mortem macroscopic and histological examinations of the injection site and of other body tissues. Although multiples of the intended human dose are administered in these studies, demonstration of lethality is usually not feasible or relevant for vaccines.

(b) *Immunotoxicity* of the vaccine dosage form, of its individual components and of its breakdown/metabolic products.

The purpose of immunotoxicity studies is to determine if the immune response elicited by the vaccine produces any adverse and undesirable side-effects. Such events could result from either expected or unexpected causes.

For example, the intention of the immunization is to produce antibodies and/or immune cells which will combine with the target antigen molecule. The removal or neutralization of the antigen by this means can result in the formation of immune complexes, which in certain forms and under certain circumstances, largely depending on the quantity of antigen and antibody present, may produce pathology in susceptible sites such as the renal glomerulus, choroid plexus, etc. In addition, the removal or neutralization of the antigen may result in disturbances to the complex systems involved in homeostasis and this risk would need to be carefully investigated before the clinical use of such vaccines could be advocated.

Although immunization with an immunogen carefully selected for its specificity to the target antigen should not produce any immunological side-effects, unexpected and unpredicted cross-reactive immunity to other, non-target, tissues may occur. Such cross-reactions may result from the hitherto unknown presence of the target molecule in tissues other than those of the intended target, or to molecular mimicry, i.e. target and non-target proteins sharing the same antigenic profile because of similarities in their amino acid sequences or as a consequence of tertiary folding of the molecules.

The choice of animal is important here as the model used must have an endogenous target similar to that of the human, and the immune response and its sequelae must also be similar to those expected clinically (see later).

Prior to Phase II clinical trials

Phase II clinical trials are carried out in fertile individuals in order to evaluate the preliminary efficacy of the method. The major objective of the safety studies to be carried out prior to this stage of clinical testing will be concerned, therefore, with determining if the use of the vaccine is associated with the development of fetal anomalies should the immunized animals become pregnant. These studies can, again, be divided into two types.

(a) *Chemical teratology* of the vaccine dosage form, of its individual components and of its breakdown/metabolic products.

It is possible that the components of a fertility-regulating vaccine – in combination or in isolation, as parent compounds or breakdown/metabolic products – may have a direct or indirect effect on the gametes, in both males or females, which results in subsequent lethal or sublethal defects in the fetus. Similarly, fetal exposure to these compounds may occur as the result of immunization of a woman

during the early stages of an undetected pregnancy or as the result of pregnancy occurring because of vaccine 'failure'.

(b) *Immunoteratology* of the vaccine dosage form, of its individual components and of its breakdown/metabolic products.

The maternal immune response elicited to a fertility-regulating vaccine might also have a teratological effect on the developing fetus, either indirectly as a result of subtle alterations in the maternal physiology on which the fetus is dependant, or directly as a result of antibodies passing across the placenta and being deposited in, or reacting with, fetal tissues. The consequences of such events may not be detectable until the offspring reach sexual maturity, in which case their fertility and the normality of their offspring might also need to be assessed.

Again, the animal species in which these studies are to be carried out needs to be carefully selected for similarity to the human in terms of its immune response to the vaccine, mode of placentation, fetal–maternal relationships, and known frequency and type of naturally occurring fetal abnormalities. If primates are considered to be the most appropriate animal models, such studies will have major time and cost implications.

Prior to Phase III clinical trials

Other safety issues, such as the nature and extent of carcinogenicity and mutagenicity studies that should be carried out with such vaccines and the stages of clinical testing at which such studies should be carried out, are less easy to define.[6,7] In view of the small quantities of vaccine to be administered and the infrequency of their administration, compared to other drugs, it may not be relevant to conduct conventional carcinogenicity and mutagenicity studies with these preparations. However, this issue is far from clear and further informed discussion is needed in this area.

It is quite possible that, as methods of detecting humoral and cellular immune responses become more sensitive and more reliable, cross-reactive immunity may be found to be associated with the use of all vaccines, irrespective of their claimed specificity for the intended target antigen (see Chapter 1). In order to develop safe as well as effective fertility-regulating vaccines, it is necessary to investigate the types, levels and long-term consequences of these cross-reactions in order to determine those that may be free of adverse side-effects and, therefore, acceptable and those that may be actually or potentially hazardous.

Efficacy studies

The same animal model requirements and restrictions that apply to safety studies with antifertility vaccines apply also to their efficacy evaluations. The animal models selected for evaluating the efficacy of a prototype vaccine will

Table 3.3. *Examples of animal models used for evaluating fertility-regulating vaccines (FRV)*

FRV	Species	Treatment	Outcome	Comments	Reference number
Antisperm MAbs	Mouse	2 i.p. injections (0.5, 1, 2 or 4 mg). M29 IgM or M42 IgG (singly or in combination)	Significant contraceptive effect singly; multiplicative when used in combination	Each sperm antigen participates in separate event in gamete interaction	9
	Baboon	0.1 or 1 mg LDH-C4 i.d. or i.m.	Reduction in fertility	Efficacy may be improved if antibody titre increased	10
Porcine ZP	Mouse	100, 250, 500, 1000 pig zonae	High titres but failure to inhibit fertility	Indicate rodents poor model	11
	Squirrel monkey (*S. sciureus*)	0.25, 0.5 or 1.0 mg PPZA; booster at 4 months	High level of antibody obtained of long-term duration	High degree of similarity of antigenic sites	12
	Long-tailed (*M. fascicularis*)	1 i.m. injection 360 μg protein; 0.25 ml boosters	High titres produced; 50% CR Aberrations in cyclicity	Side-effects may be due to impurity of zona preparation	13
	Rhesus (*M. mulatta*)	1 i.m. injection of 250 μg	Rise in antibody titres (IgG)	No change in cyclicity until 14 weeks post-treatment	14
Gonadotrophins (GT) ß-hCG	Marmoset (*C. jacchus*)	100 μg i.d. with boosters every 2.5 years	Fertility suppressed	As titres declined, CR increased followed by abortions	15
Variety of GT combinations	Bonnet (*M. radiata*)	3 i.m. injections of 50 μg equivalent of GT monthly	Animals become pregnant when neutralization capacity diminishes	Given primate model may not be suitable for efficacy studies where antibodies generated are species specific	16

ß-oLH	Rhesus (*M. mulatta*)	50 μg; boosters at 6–12 months	Reduction in fertility (2% vs 50% in controls) Antibody response varied Impaired luteal function No effect noted at necropsy	Significant cross-reaction of anti-oLHß antibody with endogenous rhesus LH	17
CTP-ß-hCG:DT	Baboon	6 i.m. injections at 28-day intervals; mated for 3 cycles	4.6% pregnancy vs. 70% in controls	Antibodies cross-reacted poorly with bCG (3–15%)	18

Note:
MAb = monoclonal antibody; i.p. = intraperitoneal; i.d. = intradermal; i.m. = intramuscular; CR = conception rate; ZP = zona pellucida; PPZA = purified porcine zona antigen; oLH = ovine luteinizing hormone; hCG = human chorionic gonadotrophin; bCG = baboon chorionic gonadotrophin; CTP = carboxy terminal peptide; DT = diphtheria toxoid.

depend, therefore, on the type of vaccine being evaluated, the antigen on which it is based, and its expected/intended mechanism of action. The same three examples, of an antisperm vaccine, an anti-ovum (ZP) vaccine, and an anti-hCG vaccine, are used to illustrate this point (Table 3.3).

Antisperm vaccines

If the human sperm antigen used for vaccine development is not species specific but is common to, and restricted to, the spermatozoa of many mammalian species, any of these animal models should be suitable for evaluating the efficacy of the vaccine. The selection of the most appropriate models would be based on similarities of their reproductive biology to the human, particularly in terms of gamete production, site of semen deposition and gamete interaction.

Some sperm-specific antigens form members of a group of related molecules which, whilst not identical, are sufficiently similar for the antisera raised to one to cross-react extensively with the others. For example, the lactic dehydrogenase isosyme isolated from mouse spermatozoa, LDH-C_4, elicits antibodies cross-reacting with the corresponding material in baboon and human spermatozoa (see Chapter 2). As a result of this cross-reaction, active immunization of female baboons with mouse LDH-C_4 leads to a significant reduction in the fertility of the immunized animals, suggesting that the baboon would be a suitable animal model in which to evaluate the preclinical efficacy of an anti-LDH-C_4 vaccine.

As an antisperm vaccine would most likely act prior to fertilization, an indication of the potential efficacy of the vaccine might be obtained by testing the ability of antisera raised to the vaccine to inhibit fertilization *in vitro*. However, such in-vitro tests need to be treated with caution and are probably only suitable for carrying out a preliminary screen of antisera raised to a range of different antigens.

Anti-ovum (ZP) vaccines

Although active immunization of female baboons with solubilized whole porcine ZP has a major effect on the subsequent fertility of these animals (see Chapter 2), such a crude preparation would not be acceptable for clinical use. It would be necessary to analyze the composition of ZP in order to identify and characterize ZP-specific molecules which could be evaluated as antifertility immunogens.

Marked differences can be discerned in the thickness and gross structure of the ZPs of different mammalian species. The extent to which these physical differences are reflected in chemical differences is not clear as studies have not yet reached the stage at which detailed comparisons can be made of the molecular components of the ZPs of different species. However, immunologi-

cal comparisons indicate that species vary considerably in the number of ZP epitopes shared (i.e. those sharing the most epitopes are the most cross-reactive). In this regard there appears to be considerable similarity in the epitopes on pig and human ZPs. As it is the ZP which appears to prevent interspecies fertilization, it is likely that the most promising antigens for development into anti-ZP vaccines may turn out to be species-specific epitopes, thus presenting major problems in terms of identifying relevant animal models in which the preclinical efficacy (and safety) of antihuman ZP vaccines can be meaningfully evaluated *in vivo*.

An anti-ZP vaccine could mediate its effect both prior to fertilization, by interfering with sperm–ZP binding, and after fertilization, by preventing blastocyst hatching. Although the ability of anti-ZP antisera to block in-vitro fertilization (as described earlier for an antisperm vaccine) and/or blastocyst hatching *in vitro* could be used for selecting the more promising ZP antigens, this would need to involve the use of human gametes and raises, therefore, major ethical questions (see Chapter 13). Recent studies, using non-viable human oocytes and isolated human ZP for the in-vitro assessment of sperm–ZP interactions, may provide a solution to this problem.

Anti-hCG vaccines

The choice of animal models for assessing the efficacy of anti-hCG vaccines is governed by the same factors as the safety evaluation described earlier in this chapter. Again, the efficacy of the anti-hCG vaccine proposed for clinical use should be compared with that of an homologous vaccine developed from the corresponding molecule in the selected animal model.

As an anti-hCG vaccine acts at the peri-implantation stage of embryonic development, the primate model selected for efficacy evaluations should be one in which the time-course, mechanism and extent of implantation are as close as possible to the human.

General observations

As described elsewhere (see Chapter 2), virtually every step in the reproductive process, from the hypothalamic releasing hormones to the differentiated placenta, is accessible to immune attack. However, from the point of view of maximizing efficacy, minimizing side-effects and avoiding immunopathology, the ideal target for an antifertility vaccine would be a molecule:

which is specific to the intended target;
which is present transiently and/or at low levels compared to the anticipated immune response;
which is present in a site where a controlled immune response will not lead to immunopathology;
which will not elicit other undesirable immune responses, such as IgE

production and subsequent allergic reactions;
which is, or can be, chemically characterized;
which, when eliminated or neutralized by immunological means, will result in a safe, effective and acceptable means of fertility regulation.[8]

If these criteria are applied, only a limited number of the possible targets for an antifertility vaccine actually represent attractive candidates for product development. This limitation should facilitate the identification and selection of the most appropriate animal models and the design of the most meaningful safety and efficacy studies to be carried out with these novel preparations.

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4 An overview of the immune system

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Introduction

The principal function of the immune system is to resist infection and, in some circumstances, to prevent the spread of malignancy, whilst at the same time maintaining tolerance to self antigens, fetal antigens, food and other harmless environmental substances. The key cells involved in the development of specific immunity are lymphocytes with receptors for antigen. The effector phase of the immune response may be mediated directly by lymphocytes or, more generally, by their molecular products which recruit and amplify a variety of innate (non-specific) mechanisms leading to inflammation, phagocytosis and killing of micro-organisms.

Lymphocytes and lymphoid tissues

Lymphocytes are derived from pluripotent haemopoietic stem cells in the yolk sac and fetal liver before birth, and in the bone marrow after birth, and are continuously replenished throughout life. They divide into two major families in the primary lymphoid organs: T cells which develop in the thymus, and B cells which mature in the bursa of Fabricius or its mammalian equivalent, bone marrow. During their maturation, lymphocytes also acquire diverse antigenic specificities by genetic rearrangement and/or mutation of their antigen receptor genes. They subsequently migrate to peripheral lymphoid tissues such as lymph nodes, spleen and mucosal aggregates as resting small lymphocytes to replace cells which have not encountered antigen and have died. Resting lymphocytes with the appropriate receptors have the capacity to undergo further differentiation and division in a primary response to antigen giving rise to a clone of short-lived effector cells and long-lived memory cells, all of which share the same receptor specificity, a process known as clonal selection. Subsequent exposure to the same antigen will result in rapid reactivation of these memory cells and the production of an accelerated and augmented secondary response.

Humoral immunity

B cells differentiate into plasma cells on antigenic stimulation and exert their effects at a distance by secreting copious amounts of antibody into the

bloodstream. As well as being able to bind to specific antigens, antibodies can trigger local effector mechanisms such as complement activation and opsonization (enhancement of phagocytosis), enabling them to neutralize and eliminate extracellular micro-organisms. However, antibodies cannot penetrate living cells and are therefore ineffective against intracellular organisms.

IgM production occurs early in the immune response, is largely T cell independent, and exhibits little or no memory. As the response proceeds, however, many B cells undergo class-switching, whereby they differentiate into IgG, IgA or IgE, producing plasma cells and memory cells; at the same time there is a progressive increase in antibody affinity due to selection amongst cells whose receptor genes have undergone somatic mutation. Class-switching and affinity maturation can only occur under the influence of helper T cells.

Cell-mediated immunity

T cells act locally at the site of antigen recognition by releasing various low molecular weight cytokines, termed lymphokines, into the tissue microenvironment. The T cell family can be subdivided into a number of subsets according to their specialized functions and surface markers. The principal effector functions of T cells are cell-mediated inflammation (delayed-type hypersensitivity, DTH) and cell-mediated cytotoxicity. Regulatory T cells are responsible for controlling the functions of other T and B cells, either by promoting activation (inducer and helper T cells), or inhibition (suppressor T cells).

Lymphocyte traffic

Memory T cells are highly motile, recirculating rapidly and continuously through lymphoid and other tissues via the bloodstream and lymphatics. They leave the bloodstream by binding to a poly-ubiquinated receptor on specialized high endothelial cells of the post-capillary venules in lymphoid organs. B cells are more sessile and are located principally in the follicular regions of the lymphoid tissues. In lymph nodes, spleen and mucosal aggregates T and B cells are segregated into separate areas, but can interact with one another at the edges of B cell zones.

Mucosal immunity

Antigen entering via the mucosal surfaces can stimulate T and B cells locally. Activated cells (blasts) travel to the draining lymph nodes where they proliferate to form effector cells which migrate back to the mucosa via the thoracic duct; memory cells can circulate between all the mucosal tissues, providing long-lasting local as well as systemic immunity. The B cells in mucosal tissues differ from those in other tissues in that a large proportion are

committed to IgA production; the latter is a specialized immunoglobulin which is released into external secretions in combination with a polypeptide (secretory component) produced by epithelial cells. IgA does not activate the complement cascade, so that whilst neutralizing common environmental antigens at the mucosal surfaces, it does not cause a harmful inflammatory response.

The immune response

The immune response is initiated when antigen comes into contact with cells of the immune system. The key events include:

antigen localization in lymphoid tissues;
antigen processing and presentation by specialized cells;
lymphocyte recruitment and activation;
proliferation of activated effector cells;
effector phase (inflammation, killing, etc.);
triggering of regulatory mechanisms;
generation of memory cells and lasting immunity.

Under special circumstances, particularly with 'self' antigens, the outcome of interaction between antigen and specific lymphocytes is tolerance rather than immunity.

Antigen localization, processing and presentation

When antigen is first encountered it travels via the lymph and/or blood to the draining lymphoid tissues (lymph nodes, mucosal aggregates or spleen, depending on the route of entry), where it is taken up and localized by specialized antigen-presenting cells (APC). In an immune host, preformed antibodies and/or memory T cells may recognize antigen at the site of entry, generating local inflammation and boosting the immune response. When recirculating memory cells encounter specific antigens they are immobilized at the site and at the same time antigen-reactive cells are depleted from the recirculating lymphocyte pool. This phenomenon is known as recruitment.

Antigen-presenting cells

The following cell types may act as antigen-presenting cells:

dendritic follicular cells (in lymphoid tissues);
Langerhans cells (in the epidermis);
mononuclear phagocytes:
  circulating monocytes
  tissue macrophages

alveolar macrophages
Kupffer cells
microglial cells
mesangial cells
synovial A cells
B cells (under certain circumstances, see below).

Antigen uptake is non-specific but may be facilitated by cell surface adhesion molecules. The efficiency of endocytosis may be greatly enhanced in the presence of specific antibody and complement which bind to Fc and C3 receptors, respectively, on the APC, a phenomenon known as opsonization. Once in the phagolysozome, antigen undergoes processing, i.e. digestion into peptide fragments which become associated with endogenous histocompatibility molecules (either newly synthesized or recycled). Antigen presentation involves the cell surface display of antigenic peptide fragments bound to self-histocompatibility molecule in a form which is recognizable by T cells bearing the appropriate antigen receptors.

Histocompatibility molecules are highly polymorphic cell surface glycoproteins encoded by the major histocompatibility gene complex (MHC). Class I MHC molecules (HLA-A,B,C) are present on all nucleated cells. Class II MHC molecules (HLA-D) are restricted in their expression principally to antigen-presenting cells and B cells, but can be induced on other cell types by γ-interferon. Although the MHC was discovered and named because of its role in allograft rejection, it is now known that the main physiological function of histocompatibility antigens is to present processed peptides to T cells. Both class I and class II MHC molecules possess a binding site to which a wide range

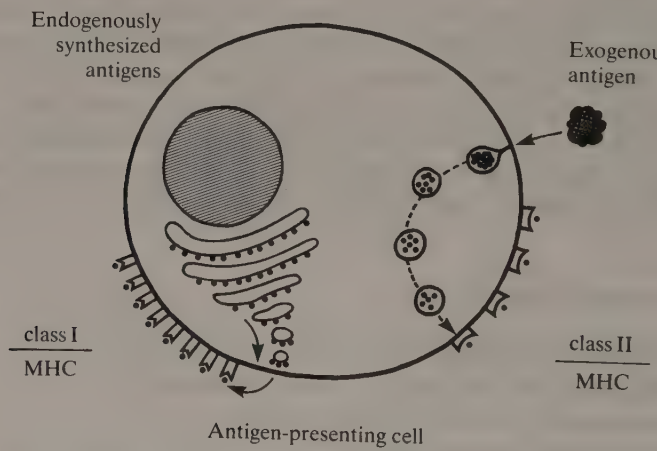

Fig. 4.1 Pathways of antigen processing and presentation.

Table 4.1. *Comparison of antigen recognition by B cells and T cells*

B cell antigen recognition	T cell antigen recognition
'Free' antigen	Cell-associated antigens
Intact cells or molecules	'Processed' antigen fragments
Conformational determinants	Linear determinants in conjunction with self-MHC

of peptides (12–18 amino acids in length) can attach. These peptides, together with surrounding portions of the MHC molecule, are recognized by the T cell receptor. The need for T cells to recognize both a self-MHC gene product and an associated foreign peptide is responsible for the phenomenon of MHC restriction of immune responses.

Different MHC molecules vary in the fine structure of their peptide-binding site, so that allelic variants are each capable of presenting a different range of antigens to the immune system. Thus, the particular combination of MHC alleles inherited by an individual may determine immune responsiveness to a specific antigen, and MHC alleles (especially class II) are therefore sometimes referred to as immune response genes. It is believed that the high degree of polymorphism amongst MHC alleles endows populations with the ability to resist and adapt to potentially pathogenic micro-organisms in a constantly changing environment.

Antigen processing and presentation may occur via two separate intracellular pathways (Fig. 4.1).

Endogenously synthesized antigens appear to be processed via the rough endoplasmic reticulum and golgi apparatus, and associate with class I MHC molecules (e.g. viral antigens, and possibly self, allo- or tumour antigens in pathological situations).

Exogenous antigens taken up into endosomes or lysosomes (as a result of endocytosis or phagocytosis and digestion) associate with class II MHC molecules.

Antigen recognition by lymphocytes

Although T and B cells are both capable of binding specific antigen via their antigen receptors, the nature of the antigenic determinants (or epitopes) recognized is different (Table 4.1). B cell surface immunoglobulins and serum antibodies bind directly to three-dimensional surface features of intact antigenic molecules or cells (conformational determinants) independent of the linear amino acid sequence. By contrast, T cell antigen receptors recognize processed antigen as a 12–18 amino acid peptide sequence, provided the latter is capable of associating with an appropriate self-MHC molecule displayed on the surface of an antigen-presenting cell.

Table 4.2. *Composition of B and T cell antigen receptors*

B cell antigen receptor	T cell antigen receptor
IgM	γ/δ^a
IgD^a	
IgG/A/E	α/β
Accessory molecules	
	CD3 complex (T cell activation)
None	CD4 (class II MHC binding)
	CD8 (class I MHC binding)
Functional changes	
Secretion	
Somatic mutation	None
Class-switching	

Note:
[a] Function unknown.

T and B cell antigen receptors

There are major differences between the molecules and mechanisms involved in the structure and function of the B cell and T cell antigen receptors (Table 4.2).

B cell antigen receptors are immunoglobulin (Ig) molecules whose diverse antigen specificities are determined by complex gene rearrangements in a set of germ-line exons encoding the variable domains. This process takes place during B cell development in the bone marrow and is referred to as the generation of diversity. Antigen receptors of different Ig isotypes are expressed in a programmed sequence by B cell progenitors, with immature cells expressing IgM alone, followed by co-expression of IgM and IgD receptors, both with the same antigen specificity. The function of surface IgD is unknown. After exposure to antigen and T cell help (see below), most B cells 'switch' to IgG, IgA or IgE synthesis. Immunoglobulins G, A and E are never co-expressed, and once a B cell is committed to one of these isotypes it cannot switch to another. Self-reactive B cells are rendered unresponsive (or anergic) by mechanisms which are as yet poorly understood.

The antigen receptor on T cells is a heterodimer consisting of either an α and β chain or a γ and δ chain, each of which has a domain structure similar to the Ig molecule. Indeed, the genes encoding immunoglobulins, T cell receptors, MHC molecules and many other cell-surface molecules all share a high degree

Table 4.3. *T cell subsets*

Subset	Markers	MHC recognition
Effector cells		
'DTH' T cells	CD4	Class II
Cytotoxic T cells	CD8	Class I
Regulatory cells		
Helper/inducer T cells	CD4	Class II
Suppressor T cells	CD4 or CD8	Class II or Class I

of sequence homology, pointing to a common evolutionary ancestry, and are known as the immunoglobulin gene superfamily. The generation of diversity amongst T cell receptors occurs in the thymus by a process of gene rearrangement similar to that of the Ig genes. Cells which recognize self-MHC (and possibly other self-antigens) with high affinity are deleted *in situ*, whilst those with low affinity for self-MHC are stimulated to proliferate and ultimately emerge from the thymus as mature T cells. T cells expressing γ/δ or α/β receptors appear to belong to separate lineages, the latter being the principal antigen receptor in 95% of fully mature peripheral T cells. The function of γ/δ receptors is unknown at present but recent data point to preferential recognition of heat shock proteins. Unlike B cells, T cells express either α/β or γ/δ receptors, but not both simultaneously.

In contrast to B cells, which can recognize free antigens, T cells can only recognize cell-associated antigens in conjunction with membrane-bound MHC molecules. To increase the efficiency of binding and activation, T cells also possess several accessory surface molecules. For example, CD4 and CD8 are responsible for increasing the affinity of binding to self-MHC molecules on antigen-presenting cells, cytotoxic target cells, and other cells with which T cells may interact. CD4 binds to class II and CD8 to class I MHC products; since CD4 and CD8 are mutually exclusive in their expression, each T cell is restricted to recognition of processed antigen in association with either class II or class I MHC molecules, but not both, and this corresponds to the different functions of T cell subsets (Table 4.3). In addition, T cells possess several adhesion molecules (e.g. leucocyte function antigens, LFA-I and LFA-2) which bind to complementary ligands (intercellular adhesion molecule, ICAM and LFA-3) on interacting cells. Another accessory structure is CD3, which is a complex of integral membrane proteins closely linked to the T cell antigen receptor and is thought to be involved in signal transduction.

T lymphocyte subsets

T cells may be divided into two broad functional categories, effector cells and regulatory cells (Table 4.3).

Table 4.4. *T cell markers*

Cluster designation	Synonyms	Function
CD1	T6, Leu6	
CD2	T11, LFA-2	Adhesion molecule SRBC-receptor LFA-3 ligand
CD3	T3, Leu4	T cell receptor signal transduction
CD4	T4, Leu3	Class II MHC ligand
CD5	T1, Leu1	
CD6	T12	
CD7	3A1	
CD8	T8, Leu2	Class I MHC ligand
CD18	LFA-1	Adhesion molecule ICAM ligand
CD23	—	Fc receptor for IgE
CD25	Tac	IL-2 receptor
CD45	T200	

The term cell-mediated immunity includes the direct actions of cytotoxic T cells as well as the cell-mediated inflammatory response (DTH) which occurs via recruitment of accessory cells. T cells which trigger B cell activation are known as helper cells and those which trigger activation of other T cells are referred to as inducer cells, although the terms are often used interchangeably.

Subset markers

A number of monoclonal antibodies are now available for identification of various surface molecules on T cells. Such molecules may be expressed only at certain stages of maturation, or selectively by one or more functional T cell subsets, or by all T cells. In some cases the normal biological functions of these molecules are known, in many cases they remain as yet unknown. Until recently, terminology was determined either by the particular monoclonal antibodies available commercially (e.g. OKT4, Leu3) or by the function of the molecule if known (e.g. Tac = T cell activation, IL-2R = interleukin-2 receptor). By international convention, these markers are now known by their CD (cluster designation) phenotype (Table 4.4). Thus, the T4 marker is known as CD4, T8 is CD8, Tac is known as CD25, etc. In clinical diagnostic laboratories, the currently most useful markers are pan-T cell markers (rosettes, CD2, CD3) which identify all T cells, and the CD4 and CD8 markers which are mutually exclusive to different T cell subsets.

Lymphokines, monokines and cytokines

Lymphokines were first described 20 years ago as 'cell-free soluble factors generated by the interaction of sensitized lymphocytes with specific antigen

and expressed without reference to the immunological specificity'. They are now recognized as a diverse family of polypeptide products released by activated T cells, and which have potent effects on a range of lymphoid and other target cells. They are secreted in minute concentrations, and until recently could be detected only by bioassay. Lymphokines were at first named according to their biological actions, but it was subsequently found that a single polypeptide could act on many different target cells giving rise to a number of effects, and resulting in great confusion in nomenclature (by 1980 there were over 100 different biological activities described).

Monokines are similar biologically active substances released by members of the monocyte/macrophage family of cells.

Cytokine is an umbrella term which can refer to lymphokines, monokines, or similar products released by any other non-lymphoid cell.

The term *interleukin* was introduced more recently to describe lymphokines and monokines without reference to the bioassays in which they were detected. By international convention, when a lymphokine is isolated, purified and sequenced, it is designated as an interleukin (IL) and assigned a number, thus: IL-1, IL-2, IL-3, etc. There are currently ten recognized interleukins.

Biology of cytokines

Cytokines are small polypeptides (15–50 kDa) which mediate the effector functions of various cell types; they have no capacity to bind specific antigen, although they are often released by cells responding to a specific antigenic stimulus; they are secreted in minute concentrations and have very potent biological activities, often acting on a variety of cell types; some of their biological activities depend on the synergistic effects of two or more cytokines; they have a very short biological half-life, and their effects are regulated by a variety of natural inhibitors or antagonists, including other cytokines (Table 4.5). In addition to antigens, their release can be stimulated by mitogens, viruses, other cytokines etc., and is controlled at the level of gene transcription. In T and B cells the coordinated release of several cytokines, as well as the expression of their receptors, plays a crucial role in regulating cellular proliferation, differentiation and function.

Cytokine receptors are found on their target cell membranes. Several have now been characterized, with the IL-2 receptor having been studied in most detail. It consists of two subunits, each of which can independently bind IL-2 with low affinity, and which together bind IL-2 with high affinity. Resting T cells express only the low affinity 75-kDa subunit, but activation results in co-expression of the 55-kDa subunit and the development of a functional high-affinity receptor. In some circumstances proliferating T cells (and their daughter cells) can both secrete IL-2 and express its receptor, giving rise to autocrine stimulation. Cytokines are very pleiotropic in their actions. One cytokine can mediate many biological effects, depending on its target cells, and one effect may be mediated by several cytokines, either in concert or singly.

Table 4.5. *Composition, source and targets of cytokines*

Cytokine	Cellular source	Cellular target
Regulatory growth and differentiation factors		
IL-1	Monocytes/macrophages, endothelial cells, B cells, fibroblasts, epithelial cells astrocytes, keratinocytes osteoblasts muscle cells	T cells, thymocytes, B cells, macrophages, neutrophils, endothelial cells, fibroblasts, hepatocytes, osteocytes, chondrocytes, epidermal cells, muscle cells
IL-2	T cells	T cells, B cells, monocytes/ macrophages
IL-4	T cells	B cells, T cells, mast cells, macrophages, haemopoietic progenitors
IL-5	T cells	B cells, eosinophils
IL-6	T cells	B cells, thymocytes
Effector cytokines		
IFN-γ	T cells, NK cells	Monocytes/macrophages, T, B and NK cells
TNF-α	Monocytes/macrophages, T cells, B cells, NK cells, thymocytes	Tumour cells, fibroblasts, macrophages, osteoclasts, neutrophils, eosinophils, endothelial cells, hepatocytes, adipocytes, chondrocytes, transformed cell lines
LT (TNF-β)	T cells	Tumour cells, neutrophils, osteoclasts, transformed cell lines
Perforin	Cytotoxic T cells	Any target cell recognized as foreign
Haemopoietic colony-stimulating factors		
IL-3	T cells	Mast cells, multipotential stem cells
IL-7	Bone marrow stromal cells	B cell progenitors
GM-CSF	T cells, endothelial cells, fibroblasts, macrophages	Multipotential stem cells
M-CSF	Fibroblasts, monocytes, endothelium	Multipotential stem cells
G-CSF	Macrophages, fibroblasts	Multipotential stem cells

Note:
Abbreviations: Il-1, 2, 3 etc. = interleukin-1, 2, 3; IFN = interferon; TNF = tumour necrosis factor; LT = lymphotoxin; CSF = colony-stimulating factor; GM = granulocyte/macrophage.

Important effects include mediation of cellular inflammatory responses, killing of target cells, regulation of lymphocyte activation, proliferation and differentiation, control of haemopoietic progenitors, and antiviral activity.

Lymphocyte activation

T cell activation

The process of T cell activation begins with the recognition of processed antigen on the surface of an antigen-presenting cell. Accessory T cell molecules also attach to self-MHC molecules on the APC surface (CD4 to class II, CD8 to class I) and increase the affinity of binding. The CD3 complex, consisting of five polypeptide chains linked to the T cell receptor in the cell membrane, is responsible for signal transduction, i.e. passing the message that antigen has been bound to the intracellular second messenger system. CD3 is thought to be coupled to a regulatory G protein through which it activates a membrane phosphodiesterase, resulting in an increase in K^+ and Ca^{2+} flux, activation of protein kinase C, and formation of inositol triphosphate (IP3).

Interleukin-1 (IL-1) released by the antigen-presenting cell, acts synergistically with the antigen-binding signal transmitted by CD3 to activate the T cell. Although IL-1 alone is not sufficient to trigger T cell activation, its absence may lead to T cell unresponsiveness. Antigen recognition, together with an IL-1 signal, induces IL-2 secretion by the activated T cell, as well as expression of the high-affinity IL-2 receptor. Thus IL-2 can act in an autocrine and/or paracrine fashion to stimulate T cell proliferation and differentiation (Fig. 4.2).

Two or three days after lymphocyte recruitment, following proliferation in the lymphoid tissues at sites of antigen localization, activated blast cells appear in the efferent lymph, thoracic duct and bloodstream, and within a week to 10

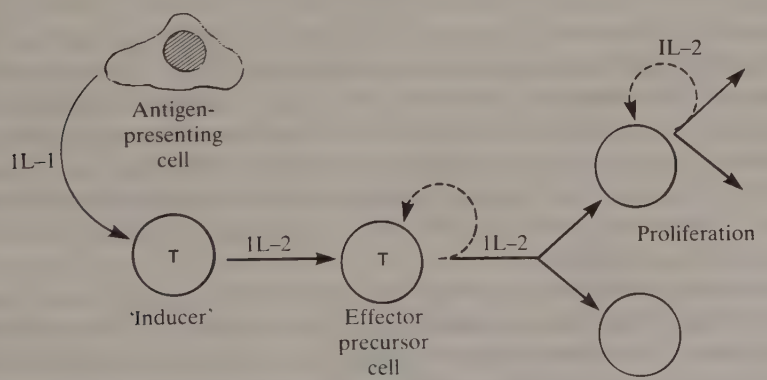

Fig. 4.2 T cell activation.

days after primary antigenic stimulation, memory cells can be found in the recirculating pool.

B cell activation

Antigen receptors on B cells (surface immunoglobulins) are linked directly to membrane G proteins and the second messenger system; simultaneous recognition of self-MHC is not required. Although direct signal transduction by antigen receptors can trigger B cells in the absence of a T cell signal, this only occurs in IgM-secreting B cells when their receptors are cross-linked by polyvalent antigens. In all other B cells, surface immunoglobulin serves not as a triggering receptor, but as a means of efficient antigen trapping for endocytosis, processing and presentation to T cells in association with self-MHC molecules on the B cell membrane.

T-independent responses and class-switching

IgM-secreting B cells can generally be activated in the absence of T cell-derived lymphokines, and IgM responses are thus said to be T independent. Some antigens, e.g. polysaccharides, are poor T cell immunogens, and stimulate predominantly IgM responses. Since memory B cell induction requires T cell help, such antigens tend not to provoke good immunological memory. However, in the presence of activated T cells, IgM-secreting B cells can be induced to alter the Ig heavy chain isotype they secrete, a phenomenon known as 'class-switching'. This results in irreversible commitment of the cell to IgG, IgA or IgE synthesis and memory induction. Reactivation of post-switch memory B cells also requires lymphokine signals derived from helper T cells.

Interactions between B cells and T helper cells

Under most circumstances, and particularly with protein antigens at low concentrations, B cells trap and internalize the antigen via their specific surface immunoglobulin receptors, process it, and present peptide fragments to antigen-specific helper T cells ($CD4^+$) in association with class II MHC molecules (Fig. 4.3). B cells thus behave in the same way as antigen-presenting cells, displaying the same antigen–MHC complex as was recognized by the helper T cell during its primary activation, and bringing the cells into intimate contact with one another.

Activation, proliferation and differentiation of B cells are regulated in a complex manner by the synergistic action of several helper T cell-derived lymphokines. IL-4 induces expression of the IL-2 receptor α chain, and IL-5 induces the IL-2 receptor β chain, so that when exposed to both, B cells are able to bind IL-2 with high affinity. Tumour necrosis factor (TNF), IL-1 and

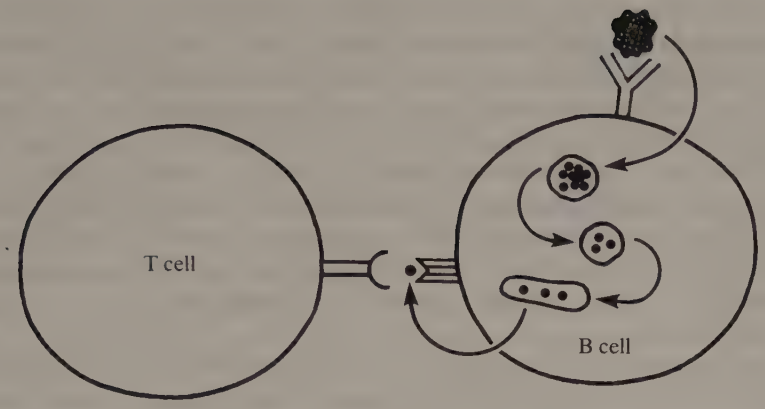

Fig. 4.3 Interaction between B cells and T helper cells.

interferon-γ (IFN-γ) can also augment B cell responses under some circumstances.

Immunoglobulin isotype switching may also be mediated (at least in part) by some of these cytokines. IL-4 is the major lymphokine involved in production of IgG_1 and IgE, and IL-5 appears to be obligatory for IgA production as determined from studies in mice.

Effector T cell function

Delayed-type hypersensitivity

Activated effector T cells can produce a cellular inflammatory reaction in any tissue where they recognize appropriately presented antigen. Although this is generally referred to as delayed-type hypersensitivity, this is a misnomer since such reactions are part of the normal protective response to infection with intracellular pathogens. In some individuals with allergic or autoimmune diseases, inappropriate T cell responses to environmental or self antigens can occur and are then properly referred to as hypersensitivity reactions. The primary cell-mediated inflammatory response takes about 10 days to develop, whereas immune individuals with circulating memory cells respond within 48–72 hours of re-exposure to antigen.

DTH reactions are initiated when $CD4^+$ T cells recognize specific antigen presented by an APC in association with class II self-MHC molecules. This, together with IL-1 released by the APC, activates the T cells which, in turn, release a variety of lymphokines as they divide and differentiate. It is these lymphokines which are responsible for development of the subsequent inflammatory reaction, particularly IFN-γ and macrophage procoagulant-inducing factor (MPIF). Tissue macrophages and passing monocytes are

induced to increase their surface expression of adhesion molecules and become immobilized at the site of the reaction, so that once the lesion is fully developed the majority of cells present are activated macrophages. The latter secrete procoagulant factors which activate the extrinsic coagulation cascade, causing plasma extravasation and further immobilization of the accumulating cells in a web of fibrin, and forming thrombin which is highly vasoactive and chemotactic. Macrophages also secrete monokines such as IL-1 and TNF, both of which are powerful inflammatory mediators with wide-ranging biological effects (see Table 4.5). Chronic antigenic stimulation can lead to formation of granulomas with multinucleated giant cells, tissue destruction and fibrosis.

T-cell-derived lymphokines, particularly IFN-γ, are also responsible for the phenomenon of acquired cellular resistance, whereby macrophages become activated and increase their capacity to kill intracellular pathogens.

Cytotoxic cells

Activated CD-8$^+$ T cells can kill virus-infected cells and other antigenic target cells by direct cell contact. Processed antigen fragments bound to class I self-MHC molecules on the target cell are bound by the T cell which then releases cytotoxic lymphokines such as lymphotoxin (TNF-β) and perforin. The latter is a pore-forming molecule which acts in a similar manner to the membrane-attack complex of the complement cascade, destroying the osmotic integrity of the target cell and causing it to swell and burst.

Other cells can exhibit cytotoxicity, but not via specific MHC-restricted antigen recognition. Killer (K) cells carry Fc receptors for IgG antibody which

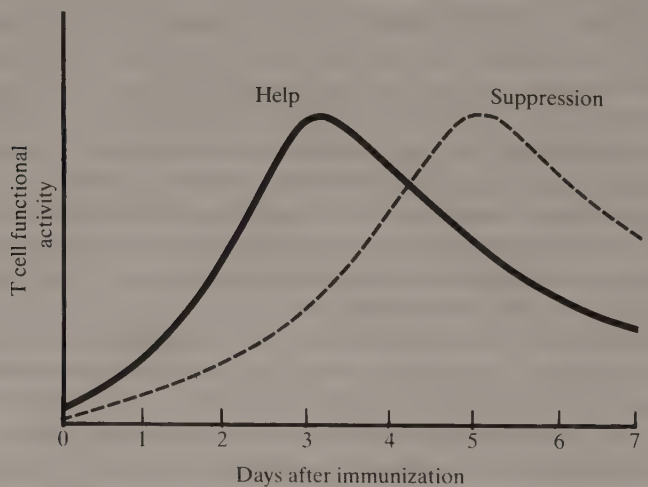

Fig. 4.4 Sequential activation of T helper and T suppressor cells.

can form a bridge between the effector cell and target cell. When contact occurs, the target cells are lysed, possibly by LT/TNF. This process does not involve the complement cascade and is termed antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC can be observed in the test tube, but its significance *in vivo* is uncertain. It may play a role in the killing of foreign cells and/or tumour cells.

Natural killer (NK) cells constitute 1–2% of the total lymphocyte pool and probably belong to the T cell lineage. *In vitro* they bind non-specifically to various tumour cell lines, which they can kill spontaneously in the absence of antibody or previous stimulation, and their activity is greatly increased by IFN-γ. NK cells play a role in the early stages of antiviral immunity before specific immunological mechanisms have been activated, and may be involved in resistance to certain tumours which express receptor sites for NK cells on their surface.

Regulation of the immune response

Suppressor T cells

T cells expressing either CD4 or CD8 are capable of inhibiting the activation of other T and B lymphocytes. Little is known of the properties or mode of action of these suppressor cells, but they appear to be responsible for down-regulation of the immune response once it has reached a peak. In some circumstances, suppressor cells can respond to antigen in the absence of conventional antigen-presenting cells, but their own activation requires interaction with a $CD4^+$ inducer T cell which has itself recognized antigen in conjunction with self-MHC.

During the course of an immune response there is sequential activation first of helper T cells, and then of suppressor T cells, with a lag phase of 2–3 days separating them (Fig. 4.4). This gives the helper/inducer T cells a chance to initiate an immune response by activating the highest affinity B cells and effector T cells, which, once stimulated, become refractory to suppression. The appearance of suppressor T cells may prevent activation of lower affinity cells, and may thus play a role in affinity maturation during the course of the immune response. It may also limit the extent of T cell proliferation, thereby promoting the reversion of activated cells to the resting state to become long-lived memory cells. Suppressor T cells thus appear to be responsible for regulating the magnitude, duration and quality of immune responses.

Network Theory

Although lymphocyte interactions are usually thought of as occurring via mutual binding of antigen, or via an intermediary antigen-presenting cell, lymphocytes can also interact in the absence of antigen by directly recognizing

the variable regions of one another's receptors. The antigen-binding site of each lymphocyte receptor (and antibody) has unique epitopes, known as idiotopes, which can potentially be recognized as 'foreign' by other cells with complementary receptors. Cells or antibodies capable of recognizing these idiotypic determinants are referred to as anti-idiotypic.

This forms the basis of the Network Theory, first put forward by Jerne in 1974. He proposed that when antigen activates a clone of cells, their proliferation stimulates anti-idiotypic antibodies which feed back and inhibit further proliferation of the clone. Each of these antibodies may, in turn, stimulate anti-anti-idiotypic antibodies which may feed back on the second wave of clones, and so on. The immune system could thus be regarded as a self-regulating network of interacting cells, with a tendency to damp down perturbations caused by the introduction of antigen. There is some experimental evidence to support this concept, but its true physiological significance remains to be determined.

Tolerance

Tolerance is the specific failure to mount an immune response, and as such is the mirror image of immunity. Like immune responses, tolerance exhibits antigen specificity and memory for the inducing antigen. Tolerance may be natural, as in the case of self-antigens or paternal antigens of the fetus, or it may be induced, either experimentally with foreign antigens or with organ transplants where the immune system is manipulated therapeutically to prevent rejection.

The mechanism of induction and maintenance of tolerance to self-antigens is still an open question. The major theories include: (1) clonal deletion, (2) clonal anergy, and (3) regulatory mechanisms including suppression. It is currently believed that clonal deletion occurs in the thymus as a means of eliminating T cells which recognize self-MHC molecules with excessively high affinity. Self-reactive B cells do not appear to be deleted, but are rendered unresponsive (anergic) by mechanisms which have not yet been elucidated. Suppressor T cells have been implicated experimentally in the induction of tolerance but their role in self-tolerance remains to be determined.

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5 Antigens and antigen presentation in relation to vaccine development

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Introduction

The aim of this chapter is threefold. The first part summarizes information about the types of antigen present in traditional vaccines against infectious diseases, the conditions of use of the vaccines and an indication of their success in controlling subsequent infections. The second part discusses more recent approaches to vaccine development. These have the potential for making possible the development of vaccines against a much wider range of diseases. As part of this approach, there is recent progress in the identification and isolation of antigens from complex sources, such as bacteria, parasites and cells. The third section of the chapter discusses briefly the need for immunopotentiation with some preparations and the prospects for achieving simplified methods of vaccine administration which would be particularly suitable for use in regions where health services are limited.

Antigens in traditional vaccines

There are about 50 vaccines in general use, mainly in developed countries, for either medical or veterinary applications.[1] They have been developed to control viral and bacterial infections almost exclusively. They fall into several categories, as follows.[2]

Viral vaccines

The commonest and most successful are live attenuated preparations, such as vaccinia, polio, measles, rubella and mumps. These give long-lasting immunity, sometimes after a single administration. Their success has been so striking that the same approach is being vigorously pursued for other viral infections. At present, there are attenuated viral preparations against eight other infections under trial, with generally encouraging results.[3] The immunological rationale for the success of these vaccines is not well documented, but it is thought that the kinetics of viral growth (production of viral antigen) may lead to persistence of antigen and a great production of long-lived memory lymphocytes, in both the T and B cell compartments. Continuing recruitment, particularly of B memory cells, provides for long-lived antibody production.[4]

There are several inactivated whole viral preparations (e.g. influenza, polio, rabies, Japanese encephalitis). Except where antigenic variation is a problem, their success rate is good; compared to infectious preparations, a larger antigenic mass and usually several injections are required.

Two subunit preparations are used. The surface antigen of hepatitis B virus (HBV) occurs free and in large amounts in the plasma of infected people. Its isolation, disinfection and use as the basis of a vaccine to prevent HBV infection constitute a modern success story.[5] Although the preparation is sufficiently immunogenic to be administered either at or close to birth, several injections are required to achieve lasting immunity.

Bacterial vaccines

Bacille Calmette–Guérin (BCG) is the only attenuated bacterial vaccine in general use. It is used successfully in infants as part of the WHO Expanded Programme of Immunization (EPI)[6] but has given variable results in adults.[7] Genetically deficient strains of Salmonella[8,9] and of *V. cholerae*[10] are being tested.

Inactivated whole organisms are of variable effectiveness as the basis of vaccines and may give significant side-effects (e.g. pertussis[11]). A subunit preparation of pertussis appears to be moderately successful in early trials.[12]

Certain subunit preparations have been outstandingly successful. Tetanus and diphtheria toxoids have very good records in infants in the EPI and they are also used in adults.[13] The capsular polysaccharides of pneumococcus and of *H. influenzae* are successfully used in adults and such preparations, conjugated to diphtheria or tetanus toxoid as a carrier, show promising results in infants.[14]

The criteria for assessing the success of most vaccines are protection from disease which could result from later exposure to the wild-type organism, with minimum morbidity and especially mortality due to vaccine administration. It has been generally accepted that formation of antibody which blocked infection (neutralization of infectivity) was the modus operandi of most vaccines[15] (except for toxoid vaccines), but this has rarely been documented and sometimes a better correlation between protection from disease and the generation of a cell-mediated immune response has been observed.[16]

Clearly, subunit preparations based on either proteins or on oligosaccharides as the antigenic moiety have been successful. Despite the continuing success of attenuated viral preparations, there is the hope that in the future, vaccines based on proteins or carbohydrates, or even simpler preparations, could be developed for a wide range of uses.

New approaches to vaccine development

T and B cell recognition

Advances in molecular biology, immunology, biochemistry and synthetic chemistry now make it possible to develop a putative vaccine based on almost any biological product provided it is of an appropriate size and is antigenic, i.e. it can be recognized by lymphocytes. This generally means that it should be predominantly composed of amino acids or sugars.

B and T lymphocytes recognize epitopes which are generally six to eight amino acids or four to five sugars in size. B cells preferentially recognize structures which have a tertiary or a quaternary conformation and which are recognized by the Ig receptors at the B cell surface; particularly with infectious agents such as viruses, these structures are often discontinuous sequences[17] and are the regions of a protein which show a high degree of hydrophilicity and/or atomic mobility.[18] Simpler structures, e.g. with a secondary conformation such as linear peptides, are also recognized.

In contrast, T cells recognize a complex at the surface of an antigen-presenting cell composed of a major histocompatibility complex (MHC) product and a peptide with a hydrophobic region which usually is an invariable sequence of the antigen. The peptides result from the action of degradative enzymes on endocytosed or synthesized proteins within the antigen-presenting cell; they may combine with class I or II MHC antigens and the complex is transported to the plasma membrane.[19,20] With the latter, there is a correlation between the capacity of an antigenic peptide to bind to purified class II MHC antigen and the capacity of an analogue peptide to inhibit accessory cell antigen presentation.

It is now generally accepted[2] that receptors on the immunocompetent T cells see the MHC–peptide complex; this recognition, together with interaction with lymphokines (interleukins) from the antigen-presenting cells (macrophage/dendritic cells), initiates the processes of differentiation and replication of the T cell to become an effector cell. In contrast, receptors of appropriate specificity on B cells interact with the 'intact' antigen; this complex is endocytosed and degraded within the B cell so that peptides may again be formed which interact with class II MHC molecules. If the complexes so formed, which migrate to the B cell plasma membrane, are recognized by already activated T helper cells, the latter secrete lymphokines which contribute to the differentiation and maturation of the B cell to become antibody-secreting plasma cells.

Thus, there are two quite different classes of antigen-presenting cells which process antigen in a similar fashion, but they have entirely different mechanisms for the initial recognition of antigen; one is highly specific and the other non-specific.

The demonstration of a specificity requirement between the MHC antigen

and the peptide means that the entire specific immune response is genetically controlled. The existence of marked immune response (Ir) gene effects[21] would therefore be expected, and variation in immune responses entirely consistent with this prediction have been observed in humans after a viral infection. A considerable effort is now being expended in devising algorithms for the prediction of these T cell sequences.[22]

The new approaches can be considered under four headings.

Synthetic antigens

In the belief that not only many components of an organism but also many regions of an important (i.e. protective) antigen of that organism were not necessary for the generation of protective immune responses, there has been a great amount of research directed towards the development of vaccines based upon oligopeptides as the critical antigenic material. At present, no such vaccine is licensed for general use, but several are being developed.

Peptide sequences may be directly determined by analysis of a protein or indirectly from the base sequence of cDNA preparations. Generally, they are synthesized by solid-state procedures and the efficiency of synthesis decreases with the increasing length of the peptide so that synthesis of peptides of 30 amino acids (30 mers) or longer becomes moderately expensive. Enzymatic synthesis of some peptides is now possible and yields a relatively cheap product if large quantities (kg) are required.

The stated advantage of a peptide-based vaccine containing one or more B cell epitopes is that of all the organism's components, the vaccine contains only those (e.g. often less than a few percent of the total) which are required to produce the specificity of an immune response.[2] Furthermore, by choosing selected sequences of a protein, other sequences which may induce immunosuppression are avoided.[23] As with any non-infectious preparation, immunosuppressed or immunodeficient people should not be at increased risk of a generalized infection. If the preparation is entirely synthetic, the risk of unwanted reactivity due to contaminants of a biological process are avoided.

The disadvantages are the requirements for:

1. possibly several T cell epitopes (to avoid Ir gene effects[24]), in the form of individual peptides, as a carrier protein or in the form of a T cell activator, such as purified protein derivative of tuberculin (PPD). Though carrier proteins such as diphtheria and tetanus toxoids are still the choice of many investigators, there remains great interest in developing entirely synthetic preparations. As well as the need for several T cell epitopes, recent work has shown a need for 'matching' particular T and B cell epitopes;[25]
2. immunopotentiators (adjuvants); and
3. multiple injections or controlled release formulations.

Both points 2 and 3 are discussed later in this chapter. It can be re-emphasized here, however, that the use of strong immunopotentiators may enhance the risk of immunopathology if the peptide has some homology with a peptide of host origin (see Chapter 1).

Antigens produced using recombinant DNA technology

The ability to isolate or to synthesize DNA coding for a particular polypeptide or protein and to transfect cells or make recombinant, live vectors so that the transformed or infected cell now makes large quantities of a particular protein, also has the potential to have a great impact on vaccine development.

The first such genetically engineered vaccine for human use made with the product of DNA-transfected cells is already marketed (the surface antigen of hepatitis B virus, HBsAg, made in yeast[5]). The production in transfected *E. coli* of proteins from other bacteria has led to the development of some successful veterinary vaccines.[26] Fusion proteins containing viral or mammalian peptide sequences attached to a bacterial protein such as β-galactosidase have considerable potential. The production of foreign proteins by yeast or by continuous cell lines[27] (CCLs) is a now a well-advanced technology. There is a wide choice of different plasmids and promoters available and high yields of the desired proteins are possible.

The advantage of protein production by transfected cells is the ability to make large amounts of a particular protein at a reasonable price and free of any donor organism contaminants. In most cases, it should not be difficult to isolate the required product in a high state of purity using techniques such as affinity-chromatography. Depending upon the cell substrate used, the protein may have the optimum tertiary conformation and so may be effectively recognized by the most appropriate B cells. The disadvantages of this approach are the possibilities of undesirable contaminants from the transformed cell, e.g. endotoxin from *E. coli* or contaminating DNA/endogenous viruses from CCLs.[27] In addition, β-galactosidase fusion proteins made in bacteria can be toxic and the complete protein made in bacteria will not be glycosylated. The additional requirements for the generation of an immune response mentioned above for peptides also apply, except that the protein itself may contain appropriate T cell epitopes.

Recombinant live vectors

The construction of live viral or bacterial vectors for DNA coding for antigens from other sources is another exciting area with great potential for vaccine development.[28] Of the potential vectors under study, four are or have been human vaccines, which means there is already considerable experience of their use. These are vaccinia and adeno viruses and the two bacterial preparations, BCG and Salmonella. On past experience, vaccinia and BCG might be used to

generate systemic immunity and in the former case, cell-mediated immunity (CMI) as well as antibody responses would occur. In contrast, adeno virus and Salmonella would be given orally and would generate mucosal humoral immunity.[29]

Detailed data on the duration of immunity given by a single administration of each vaccine are not available. Possibly because of persistence of the organism, BCG vaccination gives long-lived immunity. Protection after vaccinia administration lasts for at least several years. The duration of immunity after Salmonella or adeno virus administration is not accurately known.

Since far more work has been done with vaccinia virus, these preparations offer comparable advantages. Apart from being highly immunogenic, once a particular construction is made, a vaccine would be very cheap, easy to administer, and could be multivalent. Some would be very stable and ideal for use in developing countries. Being infectious preparations, each might cause complications in certain recipients. Detailed data on the incidence of complications after injection of vaccinia are available from the smallpox eradication campaign.[30] It is worth noting that recombinant vaccinia preparations are markedly less virulent than the parent virus.[31]

Recent work in a model system suggests that the additional inclusion in the hybrid virus of genes coding for interleukins may in addition enhance the safety of preparations for administration to immunocompromised or immunodeficient recipients.[32,33] This is a new development with a great potential for useful application. Thus, if attenuation of a live virus proceeds too far, the preparation loses immunogenicity; it would be a significant achievement if the inclusion in the vector of an appropriate cocktail of lymphocyte growth factors was found to enhance responses in general and particularly in otherwise poor responders. Thus IL-2, IL-4 and IL-5 now seem to be required to get optimal B cell activation.

Anti-idiotype preparations

The third approach is based on a theoretical concept which was later experimentally verified. It was proposed that, in the generation of an immune response to an antigen, T and B cell receptors of the appropriate specificities (resulting in part from somatic mutation) would be produced in large numbers and the specific regions of these – the idiotypes – would in turn be recognized as foreign. Antibodies recognizing the idiotypes – anti-idiotype antibodies – would be formed. Those that recognized the antigen-binding site of the initial antibody could be considered as the internal image of the original antigen and would thus be able to act as a surrogate antigen.[34] There are now many examples where anti-idiotype preparations have been used to generate a specific immune response to a pathogen (reviewed in reference 2); two such examples are monoclonal antibody to poliovirus type 1[35] and to rabies

virus.[36] It should also be mentioned that this approach may also lead to a preparation which enhances pathogenesis.[37]

A particular advantage of this approach is that it is theoretically possible to immunize against a pathogen (or substance) without either isolating or knowing very much about the pathogen or substance in question. Despite this, there is no example as yet where a vaccine has been developed using this approach in preference to the conventional approach.

This approach may have a particular use in the area of preventing or modifying autoimmune disease. There are two reports where passive immunization against class II MHC molecules has prevented or modified such diseases in model murine systems. Another report suggests that effector T cells, which on transfer in model systems can induce an autoimmune disease, may be used as a surrogate antigen to induce an anti-idiotypic response, which thus might immunize against the appearance of such effector T cells. These approaches will be discussed in more detail in Chapter 10.

Identification and isolation of vaccine candidate antigens

Traditionally, the task of establishing the identity of protective antigens in simple viruses has been relatively straightforward. It has been more difficult with complex viruses and with bacteria because of their greater complexity. To identify particular antigens in or on more complicated organisms such as parasites, with their sometimes different forms, and of cells such as sperm, ova and the early conceptus has been more difficult still. Immunofluorescence using polyvalent antisera, coupled with tests to assay the biological activity of the antisera where appropriate, was a major technique. Two developments have made and are making this task more effective. The first is the availability of monoclonal antibodies and their use in biological assays, in identifying individual proteins and, where appropriate, for the isolation and/or purification (affinity chromatography) of the protein. For example, monoclonal antibodies have been prepared which allowed the isolation of a protein which acts as a receptor in the attachment of human rhinoviruses to cells,[38] and the analysis, from different species, of the CD4 molecule which acts as the cellular receptor for the human immunodeficiency virus.[39] The second development is the establishment of gene banks of the organism or cell.[40] As, in many cases, the search will be for an antigen expressed at the surface of the organism or cell, this approach enables all such antigens to be documented.

These two developments, used together, greatly facilitate a logical search (as distinct from a hit-or-miss approach) for potential target antigens.

The need to enhance the immunogenicity of simple antigens

The term adjuvant is used to describe a preparation which, when given with, particularly, a simple antigen, substantially increases its immunogenicity.

Adjuvants may have many discrete functions but three are thought to be very important.

1. To provide a depot so that the antigen persists at or near the injection site. This was initially noted over 50 years ago when alum was first used for the purpose of delaying the disappearance of the antigen from the injection site.
2. To attract relevant cells, such as potential antigen-presenting cells and responding lymphocytes, to the antigen depot.
3. To activate such cells and so induce the production and secretion of the various factors, including interleukins, which are required for the induction of an immune response.

There is now also considerable evidence that adjuvants may vary in their ability to stimulate different responses. Thus, Freund's complete adjuvant stimulates a wide range of responses, both humoral and cell-mediated, whereas alum (still the only adjuvant authorized for medical use) mainly stimulates humoral responses. Dextran sulphate may preferentially stimulate the production of an Ig isotype which fixes complement, a very important mechanism in the control of some infectious agents.

Recent years have seen a great increase in our knowledge of the function of interleukins in this process. Many of these factors have multiple activities and may be produced by different cell types; there is still some way to go before a clear-cut picture emerges of their roles at different stages of the immune response *in vivo*. Nevertheless, adjuvant mixtures of the future may include interleukins (or genes coding for them incorporated into live vectors – see above) selected for their ability to induce particular immune responses.[2] The ability to measure the production of selected interleukins by activated immune cells is expected to become an important component in assessing vaccine efficacy (see Chapter 4).

Several aspects deserve further comment.

Controlled release formulations

Oily emulsions or inert carriers such as alum provide a depot for the release over time of the antigen. Attempts to replace such mixtures with improved formulations have generally been unsuccessful, although there are exceptions.[41,42] Perhaps the most promising development is the use of controlled release systems, such as microcapsules or microspheres, composed of biodegradable materials.[43] These can be constructed or engineered to give release of the antigen in a predetermined manner. This may be, for example, a linear release profile or a pulsed release pattern. Immunostimulatory complexes (ISCOMS) and liposomes have also shown promise in this regard.

Provision of T cell help

A major result of the activation of antigen-presenting cells is the induction of T cell help for both antibody production and other effector cell generation. The antigenic requirements are met by coupling the hapten to:

1. a large foreign protein which would be expected to have several T cell epitopes and thus reduce greatly the risk of a significant proportion of poor responders due to Ir gene effects. Tetanus or diphtheria toxoids are often chosen for this reason and also because most people would have been immunized to these proteins and thus would be expected to mount an enhanced T cell response.[14] There is the risk that some people might mount a hypersensitivity response. The core antigen of HBV has also been shown to be an effective carrier in some systems;[44]
2. a T cell activator, such as PPD;[45]
3. known T cell epitopes.[25]

Early findings with several systems suggest that a number of epitopes may need to be used to avoid variable responses. Polymerization of the peptide itself (through attached cysteine residues) has also been shown to be effective[46] but this may not be a general approach.

Antigen-focusing

Current work also suggests that there may be advantages in focusing an antigen onto specific cells involved in the immune response. These include B cells, via their Ig receptors,[47] or other antigen-presenting cells, via the class II MHC molecules on their surface.[48]

Routes of antigen administration

There are two main routes of antigen administration – parentally or via mucosal or epithelial cell surfaces. The efficacy of the different routes in the induction of different reponses is in part a reflection of different types of antigen-presenting cells, such as Langerhans cells in the epidermis. In experimental systems, intravenous or intraperitoneal injection of the antigen may induce suppression of some responses, such as delayed-type hypersensitivity (DTH), whereas linear dose responses occur after subcutaneous inoculation.

Antigen administration by the oral route for the prevention of enteric infections is well established for polio (oral polio vaccine) and seems quite promising for cholera[49] and typhoid fever[50] immunization. This route is potentially important for another reason. It was shown many years ago[51] that specific IgG antibodies to polio virus appeared in the uterine and vaginal secretions after intergastric immunization with polio vaccine. Rabbits have

been successfully immunized by anal deposition of antigen and this route of immunization is commonly used in the poultry industry.

Antigen entry at a mucosal site is of particular interest in the case of fertility-control vaccines, one being the possibility of immunizing females against sperm antigens. It is now well recognized,[52,53] at least in model systems, that lymphocytes specifically sensitized at one mucosal site circulate to other mucosal sites and may afford protection against challenge at a site distant to the site of immunization. For example, adeno virus infection is responsible for about 10% of respiratory disease. The adeno virus vaccine currently used by the US military forces is live virus in an enteric-coated capsule and is administered orally.[54] The virus is thought to replicate in the gut-associated lymphoid tissue (GALT) underlying the M cells lining the walls of the intestine; the lymphocytes so sensitized migrate to the lungs and afford protection from a respiratory infection.

The finding that oral ingestion of sperm by female rats may lead to reduced fertility[55] is highly relevant to the present discussion. It has also been shown[56] that the presence of antibodies in genital tract secretions after immunization directly into the Peyer's patches is hormone dependent; antigen-specific IgA and IgG accumulation in the uterus was stimulated by estradiol at a time when antibody accumulation in the vagina was inhibited.

These findings have reawakened interest in the possibility of devising vaccines which could be administered orally and give good immunity against a variety of agents. Generally, antigens given orally in moderate doses induce tolerance rather than immunity. Apart from the use of live vectors mentioned above, experiments have been done using adjuvants such as muramyl dipeptide (MDP)[57] and incorporation of the antigen into liposomes.[58] Though this results in increased IgA responses, there may be an undesirable side to such approaches: the administration of the adjuvant may induce an immune response to bystander antigens, e.g. from food stuffs. Some antigens given by this route are highly potent immunogens. The cholera toxin B subunit is in this class, due to its affinity for certain gangliosides on the M cells. Chemical coupling of this subunit to, or its simple addition to, other antigens causes an increased IgA response to the latter.[59,60] An alternative general approach for increasing immunogenicity specifically to a putative antigen might be to attach to it an anti-receptor (GM1) ligand.[61].

In summary, antigen presentation and delivery remain very important aspects of vaccine development programmes. Substantial advances in both areas are expected within the next few years.

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6 Assays for relevant human immune responses

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Introduction

The strategy for vaccine development to control infectious disease is to generate long-lasting (possibly life-long) immunity. Current understanding suggests that there are two requirements to achieve this.

1. The generation of large numbers of long-lived B and T memory cells, as the half-life of effector T and B cells is thought to be of the order of a few days only.
2. A mechanism for the persistence of antigen, for example on dendritic follicular cells in secondary lymphoid tissue, to ensure that at least B memory cells and possibly some T helper memory cells are continuously recruited to collaborate in the formation of antibody-secreting cells over a prolonged period of time.

In contrast, the policy adopted by the WHO Task Force on Vaccines for Fertility Regulation has been to develop a vaccine capable of inducing an immune response designed to inhibit fertility and which would wane and become ineffective after a defined period of time, say 1–2 years. The requirement for reversibility and a return to a state of fertility means in effect that the immune response generated by the vaccine should be unaffected by the periodic appearance of the target antigen in its natural form, i.e. in the absence of adjuvants. Such a goal would be more likely to be achieved if a hapten–carrier conjugate was used in which the vaccine component *per se* was the hapten expressing only B cell epitopes and the T cell response was confined to foreign epitopes on the carrier.

Immunological assessment of human subjects is a necessary part both for the pre-registration phases of antifertility vaccine testing and for post-registration surveillance. In the first place, it is important to ensure that subjects selected to receive the vaccine are not suffering from any overt immunological disorder, and that they are not at significant risk of developing an unwanted autoimmune or hypersensitivity reaction. Secondly, it is important to monitor the immunological response to the vaccine in order to help establish its efficacy and to detect any unwanted effects. Both pre-vaccination and post-vaccination assessment involves clinical as well as laboratory evaluations. Clearly, a more exhaustive assessment must be carried

out during the pre-registration phases to ensure accurate definition of the most relevant parameters for subsequent routine screening of vaccine recipients, testing for efficacy, and surveillance for unwanted sequelae.

Disorders of the immune system

To appreciate the place of laboratory assays of immune competence in vaccine programmes it is first necessary to review how the immune system can malfunction and how this can be assessed clinically. The immune system may in fact malfunction in several ways.[1] Immune responses may be deficient, or they may be excessive (or inappropriate). Such disorders may be primary, i.e. due to an intrinsic abnormality within the immune system, or they may be secondary, i.e. due to the effects of some other pathophysiological process on the immune system. Disorders of this kind may affect immune responsiveness generally, or they may involve only one arm of the immune system, or they may be specific for a particular antigen or group of antigens. In other circumstances, cells of the immune system may undergo neoplastic change, or they may become infected with lymphotrophic viruses.

Most immunological disorders can be thought of as affecting B cell function (humoral immunity), T cell function (cell-mediated immunity) or both, forming a convenient framework within which to undertake clinical and laboratory evaluations. Disorders of phagocytic function and complement components do occur but are rare and beyond the scope of this chapter.

Immunodeficiency states

Immunodeficiency results in reduced immune responsiveness and susceptibility to infection. In some circumstances there may also be an increased incidence of certain neoplasms.

Primary immune deficiencies may be due to congenital abnormalities of the lymphoid organs, to inborn errors of lymphocyte metabolism, or to abnormal regulation of lymphoid progenitor cell development.

Secondary immune deficiencies may be due to toxic, nutritional or metabolic effects on the immune system, or to loss and/or destruction of cellular or molecular elements of the immune system.

Hypersensitivity reactions

Hypersensitivity reactions are excessive or inappropriate immune responses, and may be directed to either self or foreign antigens. The immunopathology and clinical features in each case depend on the nature and distribution of the antigen, the nature of the immune response, and the effector mechanisms involved.

Table 6.1. *Lymphotropic viruses*

Cells infected	Viruses	Clinical manifestations
B cells	EBV	Infectious mononucleosis
		Burkitt's lymphoma
		Duncan's disease (immunodeficiency)
	HHV-6	Roseola
		? Other manifestations
T cells	HIV-l, 2	Acquired immune deficiency syndrome (AIDS)
	HTLV-l,	T cell leukaemia

Note:
EBV, Epstein Barr virus; HHV, human herpes virus; HIV, human immunodeficiency virus; HTLV, human T-cell leukaemia virus.

Autoimmune diseases

Autoimmune diseases occur when there is an excessive or inappropriate response to *self* antigens.

Allergic reactions

Allergic reactions occur when there is an excessive or inappropriate response to *foreign* antigens.

Lymphoid neoplasia

Lymphoid neoplasia (lymphoproliferative diseases) occur when there is uncontrolled growth of a single clone of lymphocytes. This may involve members of the B cell or T cell lineages or cells of the mononuclear phagocytic system, the clinical features depending on the cell type and stage of differentiation at which the abnormality arises.

Infections of the immune system

Infections of the immune system occur when certain lymphotrophic viruses infect lymphocytes. Clinical features depend on the nature of the virus and the host response, and may result in temporary or permanent disturbance of immunological function (Table 6.1).

Infection may give rise to an asymptomatic carrier state, an acute self-limiting illness with complete recovery or to progressive disease. The spectrum of immunological effects includes immunodeficiency states, autoimmune or allergic phenomena, and lymphoid (or other) malignancies.

Clinical evaluation of immunity

Only those considerations pertinent to screening and surveillance of vaccine recipients will be discussed here. If any immunological disorder is detected, it

may warrant more detailed clinical and laboratory evaluations, description of which is beyond the scope of the present discussion.

Immunodeficiency

B cell (humoral) deficiencies can be recognized by the occurrence of repeated infections with pyogenic bacteria and some viruses, whereas T cell (cell-mediated) immune deficiencies are characterized by infections with intracellular micro-organisms including a number of viruses, intracellular bacteria and other opportunistic organisms such as fungi and protozoa. The latter are ubiquitous organisms which rarely, if ever, cause clinical disease in the absence of an underlying immunological deficit.

The major congenital immune deficiencies are of no relevance in an adult vaccination programme. They present with severe infections in the first few months of life and such individuals rarely survive beyond infancy unless the immune deficit can be repaired. The most common primary immune deficiencies in adults are B cell disorders with reduced total immunoglobulin production or with selective deficiencies of IgA and/or IgG subclasses. These are characterized by recurrent bacterial and viral infections at the mucosal surfaces (e.g. ears, sinuses, upper and lower respiratory tract, gastrointestinal and genitourinary tracts).

T cell deficiencies are most commonly seen as a result of malnutrition, malignancy or treatment with immunosuppressive/cytotoxic drugs, or in patients with human immunodeficiency virus (HIV) infection. They should be suspected in patients with unusual opportunistic infections, or with common viral or fungal infections which are unusually extensive or persistent.

Vaccines should be administered with great caution to patients with deficient immunity. Potential problems include:

1. generalized or extensive infection with a living attenuated organism (only with live vaccines);
2. failure of the vaccine to produce the desired protective immune response;
3. increased risk of allergic and autoimmune reactions.

Autoimmunity

A vaccine designed to immunize against a self antigen, of which the β-human chorionic gonadotrophin (β-hCG) and β-hCG–carboxyterminal peptide (CTP) vaccines are examples, should not be administered to patients with pre-existing autoimmune disease or with a family history of such diseases. Possible consequences could be the inadvertent exacerbation of a pre-existing autoimmune disease, or an excessive response to the vaccine resulting in irreversible infertility.

Autoimmune diseases may be classified into organ-specific diseases (e.g. pernicious anaemia or thyroiditis) or multisystem diseases such as systemic lupus erythematosus (SLE) and polymyositis.

In organ-specific autoimmunity there is often a family history of related conditions. In the context of a birth control vaccine, any history of autoimmune endocrine disease is of particular relevance.

The family history is not usually helpful in multisystem autoimmune diseases. A history of skin and/or joint symptoms should be sought, and the patient should be questioned about Raynaud's phenomenon, dry eyes and mouth, renal, pulmonary or haematological disorders. Joint deformity, synovitis and nodules may suggest rheumatoid arthritis. Non-deforming arthralgia, photosensitivity, glomerulonephritis, pleurisy, pericarditis or neuropsychiatric symptoms point towards SLE. Autoimmune haemolytic anaemia or thrombocytopaenia may occur in isolation or as a manifestation of SLE; phospholipid antibodies, with or without other features of SLE, may result in repeated miscarriages or stillbirths, and may be associated with a thrombotic tendency.

Allergic diseases

A history of hay fever, asthma, atopic eczema, or a family history of similar diseases may help establish the presence of an atopic state, although vaccine reactions can occur unpredictably in individuals without any previous history of allergic disease. Past occurrence of urticaria, angioedema, laryngeal oedema or anaphylactoid attacks should be noted, and any evidence of allergy to specific drugs, vaccines or preservatives should be sought.

Lymphoproliferative diseases

Lymphomas and leukaemias may be manifested by enlargement of the liver, spleen and/or lymph nodes, and by anaemia or other evidence of bone marrow infiltration. Multiple myeloma and other paraproteinaemias are rare in the age group likely to be vaccinated for fertility control.

HIV infection

Screening of potential vaccine recipients should routinely include questioning about risk activities which may have resulted in exposure to HIV, and about any history of other sexually transmitted diseases or unusual infections. Physical examination should include a search for lymphadenopathy, needle marks and the characteristic lesions of Kaposi's sarcoma.

Laboratory evaluation of immunity

Tests of humoral immunity

Routine tests

Total serum immunoglobulin levels may not detect a selective B cell abnormality, and in patients with a suggestive history of recurrent infection, IgG subclass measurements should also be performed.[2]

Serum electrophoresis may show evidence of a paraprotein or, more importantly, polyclonal hypergammaglobulinaemia. The latter may occur in patients with chronic infection, liver disease, multisystem autoimmune disease and certain inflammatory diseases such as sarcoidosis. In addition the albumin level may give an indication of malnutrition, liver or renal disease.

Specific antibody assays

Specific antibody levels to be measured depend on the particular vaccine to be used. For example, pre-existing antibody levels to the organism should be measured before contemplating vaccination against hepatitis B or rubella. With antifertility vaccines it may be relevant to measure antibodies to carrier molecules such as diphtheria or tetanus toxoids, as well as to the hapten component of the immunogen. A Schick test may be helpful under these circumstances.

If deficient B cell responsiveness is suspected, the IgM response can be assessed by measuring isohaemagglutinin levels and the IgG response can be assessed by measuring a rise in antitetanus antibody titre following immunization with tetanus toxoid.

A range of different assays is available for measuring specific antibody responses. In the case of vaccine-generated responses, both avidity and concentration of the antibodies are important. Broadly speaking, the assays can be divided into two groups – antigen recognition assays, which measure the relative amount of antibody in comparison with a standard, and antigen-binding assays, which can provide an estimate of avidity as well as the actual concentrations of antibodies in immune sera.

Antigen recognition assays are based on the recognition of the antigen by the antibody, and on the formation of an antigen–antibody complex which can be measured quantitatively.

Technically, the majority of the assays are carried out in such a way that the antigen, in excess, is immobilized on a solid surface (microtitre plate, polystyrene bead, etc.) and incubated with a suitable dilution of the serum being tested. After washing, a labelled anti-immunoglobulin is attached by incubation to the antibody molecules previously bound to the antigen.

The label may either be an enzyme or a radioactive tracer, usually ^{125}-iodine. In some instances, anti-immunoglobulin is not needed, as, for example, when labelled protein A is directly bound to the antibody being

assayed. Other techniques not requiring anti-immunoglobulin are agglutination and radial immunodiffusion assays. There are many variants of the antigen recognition techniques and the main features of some of them are summarized in Table 6.2.

The assays 1–4 in Table 6.2 have a common name, enzyme-linked immunosorbent assay (ELISA). There is a variety of enzymes which can be coupled to an anti-immunoglobulin and there is an even greater variety of substrates. A reaction between an enzyme and a substrate (e.g. alkaline phosphatase and p-nitrophenyl phosphate) results in a product whose colour can be assessed by eye in a semi-quantitative assay, or by a photometer in a quantitative assay. The common feature of the ELISAs is a possibility of a high throughput of samples which may be important in clinical diagnostic laboratories or in the development of monoclonal antibodies (screening of hybridomas). Photometers of a special construction (multireaders) make it possible to measure results simultaneously from the 96 wells of a microtitre plate.

The assays utilizing radioactivity as the end-point (5–7 in Table 6.2) do not have this advantage; each tube or well has to be processed individually for measurement of radioactivity. On the other hand, these assays have the potential advantage of a sensitivity higher than that of ELISA. Furthermore, the use of ^{125}I-protein A as a universal reagent obviates the need of coupling a label to the anti-immunoglobulin.

The technically most simple assays are the radial immunodiffusion assay (9 in Table 6.2) and the agglutination assays (10, 11 in Table 6.2), none of which requires a label. While the use of the former assay has been limited owing to its low practicability, the agglutination assays have found a significant application as rapid, quantitative assays providing 'yes' or 'no' (positive or negative) answers.

In the original agglutination tests, the antigen was attached to the surface of stabilized red blood cells and, in the presence of antibodies, haemagglutination occurred. Red blood cells have recently been replaced by latex and polystyrene microbeads in a number of assays.[12–16] When the antigen (attached to microbeads) is brought into contact with the antibody, a cross-linkage occurs leading to the formation of visible aggregates. The degree of agglutination can either be measured quantitatively[12,14] or assessed by inspection.[16]

A feature common to all antigen recognition assays is the impossibility of determining the concentration of the antibody as the result of the assay proper. Therefore, results have to be related to those obtained with standard immune sera in which the antibody content is usually defined in terms of units per volume.

Antigen-binding assays depend on the premise that the concentration of an antibody (or its binding sites) in serum (or plasma) is proportional to the amount of antigen bound *in vitro* to the antibodies. This binding is 'visualized'

Table 6.2. *Antigen recognition assays for the quantitation of antibodies*

Assay Number	Solid phase	Antigen	Antibody	Anti-immunoglobulin	Label	End-point	Reference
1	MP[a]	Various	Monoclonal	Anti-mouse	β-galactosidase	Photometry	3
2	Polystyrene tube	Microbial cell lysate	Patient sera	Anti-human IgG, Igm, IgA	Alkaline phosphatase	Photometry	4
3	MP	Larval exoantigen	Mouse sera	Goat anti-mouse IgG, IgM	Horseradish peroxidase	Photometry	5
4	MP	Ovalbumin	Patient sera	Goat anti-human	Biotin + avidin with β-galactosidase	Photometry	6
5	Paper disc	Allergen	Patient sera	Anti-human IgE	^{125}I	Radioactivity	7
6	Polystyrene beads	Solubilized sperm membrane	Patient sera	—	^{125}I-protein A[b]	Radioactivity	8
7	MP	Hybridoma cell surface	Monoclonal	Rabbit anti-mouse	^{125}I-protein A[b]	Radioactivity	9
8	Cellulose nitrate discs	Various	Monoclonal	Anti-mouse IgG	Fluorescein[c]	Fluorimetry	10
9	Agarose gel	Tetanus toxoid	Patient sera	—	—	Precipitation rings	11
10	Tanned red cells	Tetanus toxoid	Patient sera	—	—	Agglutination	11
11	Latex beads	Allergen	Patient sera	—	—	Agglutination	12

Note:

[a] Microtitre plate.

[b] Protein A: a constituent of the cell wall of *Staphylococcus aureus* which binds to the Fc region of human or rabbit IgG antibodies; ability to bind to mouse or rat IgG is limited.

[c] A reversed system can also be used i.e. anti-mouse IgG is bound to the solid phase and antigen is labelled with fluorescein.

by labelling the antigen, usually with ^{125}-iodine. When equilibrium between the bound and unbound antigen has been reached, the bound fraction is separated and its radioactivity is measured.

Comparative data are obtained when various dilutions of each of the antisera to be tested are incubated with a fixed amount of the labelled antigen, and binding versus dilution curves for individual antisera are constructed. A comparison of antibody concentrations can be obtained, for example, by extrapolating the dilutions required for 50% binding in individual serum samples. It is obvious that such data are of a comparative nature only, as they are dependent on tracer concentration and other assay conditions.

Data of an *approximative* nature are obtained if a single dose of labelled antigen is used with that concentration of antiserum which preferably gives a relatively low binding, e.g. the ratio of bound to total radioactivity is less than 0.3. The concentration of the bound fraction (calculated per volume of antiserum) is then considered to equal the antibody concentration. However, even if two or more antisera dilutions are used and the data are averaged, the results may be biased because they cannot be given full mathematical and statistical treatment with respect to the law of mass action (see below). Obviously, one-point measurements cannot be avoided if the serum volume and/or the antibody concentration are very low.

If this is not the case, a *quantitative* assay can be carried out by incubating a suitable serum concentration with increasing concentrations of the antigen, by using either the labelled species alone, or a constant concentration of the labelled antigen with increasing doses of the non-radioactive standard antigen. At all concentrations of antigen, the concentration of antibodies has to be in excess, except for one large (e.g. 100-fold) dose of antigen which is used for the estimation of non-specific binding. However, serum withdrawn before immunization can be used for the same purpose.

In the calculation of results, a model based on the law of mass action can be applied. This model can yield not only the correct estimate of the concentration of the antibody, but also of the antibody avidity by providing an estimate of the equilibrium (association) constant (K). If the number of different antigen concentrations in an assay is sufficiently large (six to eight), it can be ascertained whether or not the antiserum contains one or two families of antibodies. If the latter is the case, concentrations and equilibrium constants of the individual antibody families can be obtained.

For the presentation of data, various types of linear plot derived from the law of mass action are available, such as the Lineweaver–Burk (double reciprocal) plot, Woolf plot, or direct linear plot.[17] In the case of a single family of antibodies, the data form a straight line whose intercepts with the axes of rectangular coordinates provide the estimates of antibody concentration and equilibrium constant, respectively.

The most popular plot is that described by Scatchard.[18] Scatchard plots

have been used for the measurement of antibodies in a large number of investigations.[19,20]

For the construction of a Scatchard plot, the ratio of the bound to unbound (free) antigen (B:F), as well as the molar concentration of the bound antigen (B) are computed for each concentration of the antigen. The ratio B:F is then plotted on the y-axis and B on the x-axis. The straight line connecting individual observations intersects the x-axis at a point indicating the molar concentration of the antibody (antibody-binding sites). The slope of the straight line is equal to $-K$. Frequently, however, it happens that a curve rather than a straight line is obtained.

This curve can be resolved into two straight lines, one being parallel with the x-axis and indicating the magnitude of non-specific binding, the other straight line describing the antibody. Sometimes, however, the curve cannot be linearized by this procedure, indicating the presence of two or more families of antibodies. Such a curve can nevertheless be resolved into straight lines as well, so that estimates of individual concentrations and equilibrium constants are obtained. This can be done either graphically[21] or with use of a computerized analysis of the data, e.g. by the programme *Ligand*.[22]

Assay sensitivity is an important feature of every assay. In quantitative assays, the sensitivity can be defined as the lowest molar concentration of antibodies in immune serum that is significantly different from the concentration of non-specific components. However, for many of the techniques described, data on sensitivity are not available.

In antigen recognition assays, the sensitivity can be determined if the antibody is purified and defined. A sensitivity of 0.5 pmol/l of IgE has been reported for a latex agglutination assay of anti-allergen antibodies.[12]

In antigen-binding assays, the sensitivity can be expressed in terms of the lowest detectable dose calculated from the radioactivity counts that significantly differ from non-specific counts. In an antigen-binding assay of anti-hCG antibodies carried out recently in the laboratories of one of the authors (S. Cekan), the lowest detectable concentration was estimated to be approximately 10 pmol/l serum.

In qualitative assays, the term sensitivity is often defined as the ability to discern true positive from negative results. In this case, the sensitivity is expressed as a proportion (percentage) of the positive observations in all truly positive observations.[15,16] To establish the true observations, results obtained with another well-established technique, such as Western blot analysis,[15] may be used.

In conclusion, it can be seen that there is a great variety of methods for the quantitative measurement of antibodies in immune sera. The most practicable assays seem to be the ELISAs followed by the assays based on labelled protein A. Additional advantages of the ELISAs are the possibility of measuring concentrations of various Ig isotypes (IgG, IgM, etc.), and the stability of the labelled anti-immunoglobulins. These assays, however, can provide infor-

mation on the concentration of antibodies only indirectly, by comparison with standard immune sera.

'Absolute' measurements are obtained in the quantitative antigen-binding assays, together with information on antibody affinity. Binding assays are, however, time consuming and require larger volumes of sera. Hence, an ideal combination for routine antibody assays would be a quantitative definition by a binding assay of an immune serum which could then be used as the standard for a recognition assay.

Qualitative (yes or no) assays, similar to those based on latex agglutination and recently described for the detection of anti-HIV antibodies, are likely to be of great interest for the future, both for laboratory use and for self-testing.

Tests of cell-mediated immunity

Routine tests

The simplest and most useful test of T cell function is to perform *delayed-type hypersensitivity (DTH) skin testing* with a panel of commonly encountered microbial antigens.[2] One or more positive reactions at 48–72 hours indicates the presence of circulating memory T cells capable of responding to the relevant antigen(s). The test also assesses the capacity of skin Langerhans cells to process and present antigens to T cells, as well as demonstrating the individual's capacity to mount a normal cellular inflammatory response. One commercially available kit includes tetanus, diphtheria, streptococcus, tuberculin, candida, trichophyton and proteus, as well as a glycerine control, and readings can be standardized for a given population. Since most individuals within a community have been exposed to one or more of these antigens, an absent or inadequate response indicates a high probability of T cell deficiency. The capacity of patients to mount a primary T cell response can be assessed by skin sensitization with dinitrochlorobenzene (DNCB) over a 10-day period. In addition, the level of reactivity to the carrier component of an antifertility vaccine can be assessed in this way.

Circulating T cell numbers and subsets (e.g. CD4 and CD8 T cells, see Chapter 1) can be measured, but this requires sophisticated laboratory equipment and stringent quality control usually only available in specialized centres. In patients with HIV infection the number of CD4+ cells may give a valuable indication of the degree of T cell deficiency.

Proliferation to T cell mitogens such as concanavalin A (Con-A) or phytohaemagglutinin (PHA) can provide a general assessment of T cell responsiveness, but is non-specific. It may be reduced if there is a generalized T cell deficiency (e.g. due to malnutrition, HIV infection or immunosuppressive drugs), but may also be abnormal during acute infections, inflammatory reactions and other circumstances where circulating T cells have been recruited to disease-affected tissues.

Tests of specific T cell responses

Specific T cell function is measured by in-vitro stimulation with antigen. The readout is usually of *proliferation* by incorporation of tritiated thymidine, but following recent advances in cytokine research the opportunity now exists to assay the synthetic capability not only of T cells but of monocyte/macrophages as well (see next section). In-vitro tests of this kind are not necessary for routine screening but may be useful if DTH skin tests are equivocal or in cases where there is a history of delayed hypersensitivity reactions to a vaccine component. In the case of proliferative responses, the culture technique can be modified to determine precursor frequencies which are of particular interest when assessing efficacy of defined antigens and peptides as vaccines.

Cytokine tests

Cytokines are inducible, heterogeneous peptide mediators that exert specific, receptor-mediated effects on target cells (paracrine action) and/or on the mediator-producing cells themselves (autocrine action). There are at present four classes of intercellular factors implicated in immune responses: *interleukins*, *interferons*, *tumour necrosis factors* and *colony-stimulating factors*. Other growth factors may also modulate immune functions (see Chapter 1).

Cytokines were first identified, then quantitated, through their biological activity. The methods using a biological response (e.g. the proliferation or the lysis of a target cell line) have several disadvantages due to the complexity of the system, including difficulty in standardizing the culture conditions and interference by inhibitors or potentiators accompanying the cytokine to be assayed. In contrast, immunological methods for evaluating the presence of cytokines rely on the recognition, by specific monoclonal or polyclonal antibodies, of the cytokine itself as a molecular entity. This mode of evaluation is more specific, sensitive, accurate and reproducible than the biological methods, and is performed more easily. In most circumstances, bioassays and immunoassays may prove complementary rather than mutually exclusive. The reagents for immunological assays are now available, but their application in the domain of fundamental and clinical immunology remains to be determined. The principles of the assays for cytokines are illustrated schematically in Figure 6.1.

Cytotoxicity tests

A number of cytotoxicity mechanisms have been identified that may be of importance in antimicrobial immunity, tumour immunity and possibly autoimmune disease.

These are mediated by cytotoxic T cells, NK cells and K cells (see Chapter

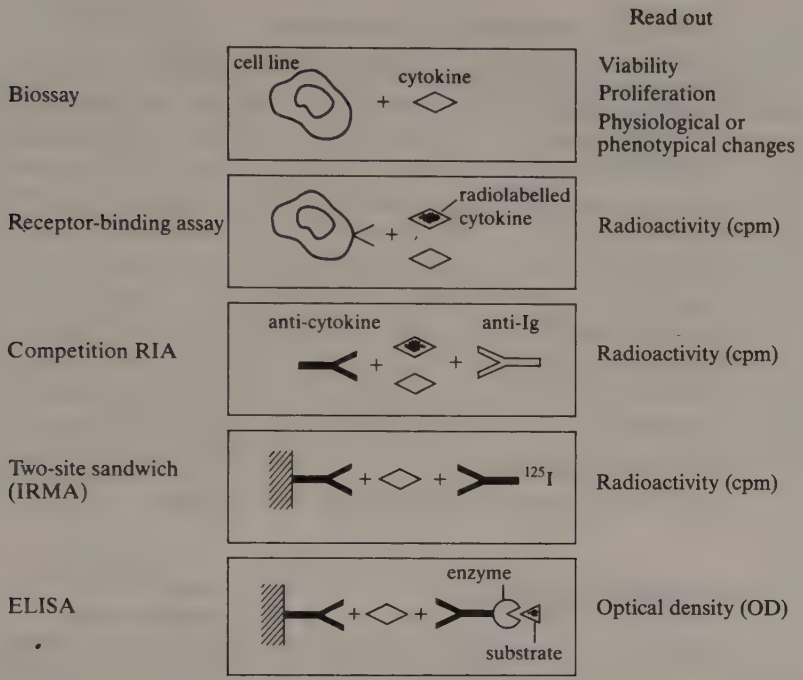

Fig. 6.1 Principles of cytokine assays.

1), assays for which are available. However, they are rarely used for routine assessment of immune function at present and remain the province of the research worker. In the case of antifertility vaccines, little is known about the possible role of cytotoxicity mechanisms in mediating their antifertility effects, although it is now recognized that antibody-dependent cell-mediated cytotoxicity (ADCC) against pre-implantation blastocysts could be worth investigating in hCG-based vaccines.

Tests of antibody-mediated immunity

Screening for allergy

Total IgE levels and skin prick tests to common environmental allergens may help to determine atopic status. If skin testing is not possible, allergen-specific IgE can also be detected by the more expensive radio-allergosorbent test (RAST) on a serum specimen.

Patients with a history of possible allergic reactions to a vaccine may be tested for specific IgE with diluted vaccine components by skin prick and intradermal testing if relevant.

Table 6.3. *Screening tests for autoimmunity*

Non-organ-specific autoantibodies:
antinuclear antibodies
rheumatoid factor
mitochondrial
Organ-specific autoantibodies:
thyroid
adrenal
ovarian
pituitary
pancreatic islet cells
parathyroid
gastric parietal cells
Complement levels:
C3, C4
total haemolytic complement

Screening for autoimmunity

Tests for autoimmunity include screening for non organ-specific antibodies, organ-specific antibodies and abnormal complement activities (Table 6.3).

Autoantibodies

Anti-nuclear antibodies are detected by indirect immunofluorescence with a tissue substrate such as rat liver, or the HEp-2 cell line. If the test is positive, or if indicated by the clinical picture, specific tests for autoantibodies to DNA and other extractable nuclear antigens can be performed.

Rheumatoid factor (autoantibody to immunoglobulin) can be detected by agglutination of IgG-coated particles or by radioimmunoassay (RIA) or ELISA. Low-titre autoantibodies can be seen in healthy individuals or during the course of acute infections and results must therefore be interpreted in the appropriate clinical context. Organ-specific autoantibodies are generally detected by indirect immunofluorescence on composite block tissue sections and, depending on the availability of suitable tissue, can be screened for easily. Some autoantibodies, e.g. against the acetylcholine receptor, involve more complex assays and need only be performed if there is a specific clinical indication.

Complement levels

Certain genetic deficiencies in individual complement components (e.g. C4) can predispose to development of autoimmunity, particularly SLE. Low levels of C3 are an indication of active immune complex deposition.

HLA typing

Immune responsiveness to many antigens is influenced by genes of the major histocompatibility complex (MHC), presumably reflecting the importance of histocompatibility molecules in antigen presentation. In humans, many immunological disorders occur with increased frequency in association with particular HLA alleles, i.e. are in linkage disequilibrium. Although HLA typing is not of value as a screening test for predisposition to autoimmunity, it may be a useful marker to record during pre-registration trials of a new vaccine in order to make correlations with responsiveness to the vaccine or with unexpected immunological reactions (hypersensitivity or autoimmunity). For example, it may be possible to predict the duration of immunity and therefore the intervals between boosters for long-term protection or the uninterrupted maintenance of effective birth control. HLA status is likely to be of particular relevance in responses to peptide vaccines which could display marked MHC restriction.

Future approaches and needs in the assessment of the immunological efficacy of fertility-regulating vaccines

In the design and performance of tests to measure immunological responses to vaccine preparations in experimental and human hosts, the determination of specific antibody levels to the antigen has been considered of paramount importance. There is little reason to think this will change. As the testing of vaccine candidates moves to Phase 2 and Phase 3 clinical trials, and later to post-registration surveillance, decisions will need to be made on a number of aspects, including the following.

1. What additional information may be required to assess, more completely, the immune response of trial participants?
2. As increasing numbers of humans are vaccinated, current methods of assessing the critical immune response(s) may need to be replaced by simple, rapid and robust screening assays which give a yes/no answer as to whether an effective immune response, e.g. a given level of specific antibody, has been achieved by a predetermined time after vaccination.
3. Increasing emphasis will need to be placed on achieving prolonged (i.e. for 1–2 years) immune responses following a single administration of the vaccine.
4. Particularly in the case of peptide-based preparations, what can be done to increase the immunogenicity of the preparation? Several approaches are possible.
5. Should a requirement arise for vaccination to induce long-lasting

immunity (i.e. vaccination would be largely irreversible), what approaches are available to achieve such responses?

6. In the case of vaccines aimed at antigens of gametes, vaccination by a mucosal route, e.g. by oral administration, may be advisable. What further research needs to be done to establish the effectiveness of mucosal versus parenteral administration of such vaccines?

These points will be briefly discussed in turn.

Measuring additional immune responses

As stated earlier in this chapter, T cell responses should be confined to the carrier component of the vaccine preparation. In the case of a peptide preparation, such as the β-hCG–CTP–diphtheria toxoid conjugate, T cell responses (blast cell transformation) to the whole conjugate, to the free peptide and to the peptide linked to an unrelated protein such as keyhole limpet haemocyanin (KLH) could be measured as a first step. When larger polypeptides, such as the β-subunit of hCG, are used, the same approach could be adopted and would yield an estimate of the extent of T cell recognition of the self or self-like component. If there was a strong T cell response, it would be desirable to assay for regulatory (Th, Ts) and for effector (Tc, Td) responses. Td responses could be measured by skin test or by *in vitro* correlates and Tc cell responses by stimulation of cells *in vitro* and by testing on sensitized target cells, as in the assessment of responses to viral infections.[23]

Such assays should preferably be initiated at the time of Phase 2 clinical trials.

The development of rapid screening assays

As increasing numbers of people participate in vaccine trials, amounting to hundreds (Phase 2 trials), thousands (Phase 3 trials) and tens of thousands (Post-registration surveillance), there will be a need for a simple screening assay to detect the desired level of specific antibody. Optimally, such an assay should be applicable to field conditions in the developing countries and should meet most, if not all, of the following criteria:

(i) rapid execution (a matter of minutes) and giving a clear-cut endpoint, easily read by both the users and providers of the vaccines;
(ii) easy to perform, with readily available, simple equipment and requiring only one or two reagents which should be heat stable;
(iii) inexpensive;
(iv) have a sensitivity at least equal to an ELISA assay;
(v) require only a few (10–20) microlitres of blood (from a finger prick) and preferably not require the separation of red blood cells (RBC) from plasma.

Although tests of this general nature have been available for some time, the advent of the AIDS pandemic has greatly stimulated their further development. Recently, tests have been described which rely on agglutination of antigen-sensitized latex[12–16,24] or gelatin beads or autologous erythrocytes.[25] These tests generally have comparable sensitivities but vary in other respects such as ease of reading. However, they might serve as a starting point for developing screening tests for detecting antibody responses to putative antifertility vaccines.

Prolonging immune responses to vaccines

Procedures for prolonging immune responses to proteins or similar preparations depend upon two factors.

(i) A form of presentation which enhances the generation of T and B memory cells. There is at present little evidence to suggest the best means of achieving this, other than by mimicking the way in which this occurs in an acute infection.
(ii) Persistence of antigen and protection from proteolytic degradation.

Precipitation with alum, first used over 50 years ago and still the only adjuvant generally used in human vaccination, achieves both of these objectives. Several other formulations used widely in experimental work, such as stable water/oil emulsions, have similar functions. The encapsulation of antigen in microspheres/microcapsules composed of biodegradable materials offers a third advantage – the release of antigen under more controlled conditions, so that, for example, a steady or a pulsed pattern of antigen release is obtainable.[26]

There may also be the opportunity in the case of peptide-based vaccines to enhance antigenicity or immunogenicity by two other approaches:

(i) decreasing susceptibility to enzymatic degradation by protecting susceptible bonds: particular amino acid residues might be substituted with d-amino acid residues;
(ii) by stabilizing the peptide in its native conformation: this has been achieved in some cases by disulphide bond[27] or other linkages.

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7 Endocrinological hazards associated with human immunization with self or self-like antigens

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Introduction

The principle of fertility regulation by immunization against factors involved in the normal process of reproduction is well established. In the case of human chorionic gonadotrophin (hCG), the approach is of proven efficacy in relevant animal models such as certain infra-human primates. All other things being equal, immunization against reproductive hormones would be a very satisfactory approach to long-term birth control within certain populations. The problem, of course, is that immunization against self-antigens may have undesirable side-effects which partly or totally exclude the use of such vaccines as contraceptive agents. The purpose of this review is to discuss the potential endocrinological hazards of various contraceptive vaccines.

Endocrine control of conception in women

The hormonal control of reproduction has been reviewed in detail in Chapter 2 of this volume and a knowledge of the processes involved therein will be assumed for the purposes of this chapter.

The endocrine milieu of early pregnancy

A wide variety of well-characterized pregnancy-associated proteins appear in the maternal circulation from the time of implantation of the embryo. Although several of these proteins were originally described as being specific to the placenta, this specificity must now be considered as quantitative rather than qualitative. The demonstration of placental proteins at relatively high concentrations in seminal plasma[1,2] and follicular fluid[3] clearly shows that these materials are not unique to the placental trophoblast.

Placental and pregnancy-associated proteins may be classified into three groups in order to provide a simple conceptual framework for what may otherwise become a topic of extraordinary complexity[4] (Table 7.1).

Group 1 products include materials which are classically regarded as specific to the placenta. Their enzymatic and hormonal activities would be expected to occur at a site distant from the placenta. The biological effects of Group 1 products, as ascertained by in-vitro studies, are not necessarily

Table 7.1. *A classification of human 'placental' products based on a variety of biological and clinical characteristics. Group 3 products, though originally isolated from placental extracts, are synthesized by the maternal endometrium/decidua. The classification does not include materials of doubtful specificity such as 'early pregnancy factor'[5] or materials characteristic of other tissues which are nevertheless found at relatively high levels in fetal/placental tissues (e.g. interferon alpha[6])*

Group 1	Group 2	Group 3
Heat-stable alkaline phosphatase (HSAP)	Placental protein 5 (PP5)	Placental protein 12[a] (PP12)
Cystine aminopeptidase (CAP; oxytocinase)	Pregnancy-associated plasma protein A (PAPP-A)	Placental protein 14[a] (PP14)
Oestrogens (E)		
Progesterone (P)		
Human chorionic gonadotrophin (hCG)		
Human placental lactogen (hPL)		
Placental growth hormone (pGH)		
Schwangerschaftsprotein 1 (SP1)		

Note:
[a] There are many synonyms for these materials.

reflected by essential functions in the pregnant woman. The fact that pregnancies may be substantially normal in the complete absence of one or other of these products virtually excludes a vital biological role during gestation. However, hCG is widely regarded as being essential to the maintenance of the corpus luteum, and therefore of an early pregnancy.

Group 2 products include placental protein 5 (PP5) and pregnancy-associated plasma protein A (PAPP-A) (Table 7.1). These products have several common features in that their suggested activities (anticoagulant[7] and anticomplementary[8] respectively) would be exerted locally at the placental site. Whether or not their activities are essential is as uncertain as for most of the Group 1 products. Group 2 products appear to behave as rather weak versions of Group 1 products, having an overall relationship to the viability of the trophoblast.

Group 3 products are not specific to pregnancy but show a striking rise in maternal blood during gestation. They are produced by maternal tissues such as the liver and/or the endometrium/decidua. Proteins of endometrial/decidual origin may have a specific function in respect of implantation and early placental formation. The rise in concentration of Group 3 products during pregnancy is probably a secondary phenomenon resulting from the production of specific Group 1 materials. Steroids are likely to be the most

Table 7.2. *The potential disorders of pituitary gonadal function in the human female which might result from immunity to reproductive hormones*

	Type 1	Type 2	Type 3
Cause	Complete failure of pituitary–gonadal axis	Relative excess of luteinizing hormone	Relative excess of follicle-stimulating hormone
Clinical features	Post-menopausal state	Anovulation, oligomenorrhoea, polycystic ovaries, hirsuteness and obesity,[a] endometrial hyperplasia	Anovulation, endometrial hyperplasia, disorders of menstruation (irregular and/or excessive bleeding)
Long-term effects	Atrophy of vagina and breasts; osteoporosis; cardiovascular disease		Endometrial carcinoma

Note:
[a] A condition often referred to as polycystic ovarian syndrome.

important controlling factors. PP14 shows a very rapid rise in early pregnancy, even before hCG, and may have a unique role in the first trimester.

Clinical disorders of the pituitary–gonadal system

There is a potentially very wide range of disorders which might result from immunity to reproductive hormones and other antigens in the human. In the absence of specific experimental evidence, it has to be assumed that these disorders would mimic, in whole or in part, the disorders which arise from pathological abnormalities of the reproductive endocrine system. A very simplified classification of these disorders is presented in Tables 7.2 and 7.3 and can be used as the basis for predicting some of the likely effects of specific immunization.

In the female (Table 7.2) the disorders have been classified into three general types. Type 1 disorders represent a complete failure of the pituitary–gonadal axis. This may occur in pathological states such as destructive intracranial tumours. It is also the effective result of the complete cessation of ovarian activity in the post-menopause, albeit this is associated with increased production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Type 2 disorders are a complex and heterogeneous range of conditions some of which were once referred to as the Stein–Leventhal syndrome. The basic cause is difficult to identify and has variously been attributed to the hypothalamus–pituitary, to the adrenal gland, and to the ovary. However, the

Table 7.3. *The potential disorders of pituitary gonadal function in the human male which might result from immunity to reproductive hormones*

	Type 1	Type 2	Type 3	Type 4
Cause	Complete failure of pituitary-gonadal axis	Relative androgen excess	Relative androgen depletion	Reduced LH/FSH
Clinical features	Feminization, impotence, loss of libido, azoospermia	Oligo/azoospermia, weight increase, greasy skin, polycythaemia, gynaecomastia	Oligospermia, feminization, loss of libido, impotence	'Normal' androgens, oligospermia, normal male characteristics
Long-term effects		? Increased risk of prostatic cancer and hyperplasia		

Note:
LH = luteinizing hormone; FSH = follicle-stimulating hormone.

common denominator appears to be a relative excess of LH, often associated with increased levels of androgens. Type 3 disorders are again complex and often of doubtful basic aetiology. In many cases, especially the peri-menopausal disorders, it is often difficult to demonstrate any endocrine abnormality other than the sequela of anovulation. However, in some cases it has been suggested that a relative excess of FSH is the main operative factor.

In the male (Table 7.3) the disorders can be classified into four types. By contrast to the female, the basic pathology and clinical findings are relatively easy to interpret. Both Type 1 (total failure of the pituitary–gonadal axis) and Type 3 (gonadal failure) lead to a general failure of reproductive function. This may include not only loss of male characteristics but also appearance of female characteristics due to the unopposed action of low levels of oestrogens. The Type 2 disorders of androgen excess are rare and result from androgen secretion by testicular and adrenal tumours.

The effects of immunity to reproductive hormones

The effects of immunity are mediated either by antibodies (humoral immunity) or immune cells (cell-mediated immunity). For the purpose of the present discussion, it will be assumed that antibodies will be the more important component in the immune response elicted by antifertility vaccines.

Antibodies to a hormone might act *in vivo* at one or more of three levels: at the cell of synthesis; in the circulation; or at target cells. It is commonly assumed that the action will be in the circulation, resulting in neutralization of the biological effects of the molecule. However, potential cytotoxic effects at the tissue level must not be overlooked since these might represent some of the most significant risks of immunization to self-antigens. For example, antinuclear antibodies have been demonstrated in the serum of mice immunized with oestradiol. This is thought to be the result of immunization with a ligand which binds to a nuclear receptor.[9] Circulating immune complexes and arterial changes have also been shown in monkeys after long-term immunization with LH.[10]

The endocrine effects and side-effects of antibodies to reproductive hormones

For obvious reasons, the majority of information on the endocrine effects of antibodies to reproductive hormones is derived from animal studies. Part of the very extensive literature on this topic is summarized in Tables 7.4 to 7.11. It should be emphasized that the aim of much of this experimental work was to explore endocrine physiology rather than immunological methods of contraception. Other aims included attempts to devise simple alternatives to surgical castration in animal husbandry.

The effects of immunity to reproductive hormones and other antigens will

Table 7.4. *Some observations on the effect of immunization of male animals with gonadotrophin-releasing hormone (GnRH)*

References	Species	Findings
Fraser & Gunn (1973)[11]	Rat (M and F)	Inhibition of gonadal function
Fraser *et al.* (1974)[12]	Rat	Suppression of LH, FSH and testicular function
Fraser & Sandow (1977)[13]	Rat	Suppression of LH and FSH; gonadotrophin release restored by active analogue of GnRH
Hodges & Hearn (1977)[14]	Monkey (M and F)	Suppression of gonadal function
Chappel *et al.* (1980)[15]	Monkey	Suppression of LH and T, returning to normal as antibody levels declined
Lincoln *et al.* (1982)[16]	Deer	Premature shedding of antlers, reduction in testicular size and circulating T, absence of rutting behaviour
Vogel *et al.* (1983)[17]	Rat	Single injection of anti-GnRH at 5 days of age leads to permanent reduction of testicular growth and Sertoli cell function
Falvo *et al.* (1986)[18]	Pig	Suppression of LH, T and testicular function
Sakai *et al.* (1988)[19]	Rat	Suppression of elevated LH and FSH and decreased mitotic activity of gonadotrophins in castrated rat
Awoniyi *et al.* (1988)[20,21]	Boar	Suppression of LH, T and testicular function; severe damage to seminiferous epithelium
Jayashankar *et al.* (1989)[22]	Rat	Suppression of T and testicular function; atrophy of prostate
Chase *et al.* (1988)[23]	Sheep	Suppression of LH and testicular function; restored by infusion of LH
Ladd *et al.* (1988)[24]	Rat/rabbit	Suppression of spermatogenesis; libido but not fertility restored by administration of T

Note:
LH = luteinizing hormone; FSH = follicle-stimulating hormone; T = testosterone.

be discussed under five headings: gonadotrophin-releasing hormone; pituitary gonadotrophins; gonadal steroids; other reproductive antigens; and early pregnancy factors.

Antibodies to gonadotrophin-releasing hormone

As would be expected, neutralization of gonadotrophin-releasing hormone (GnRH) leads to a failure of the hypothalamic drive to the pituitary, reduced gonadotrophin secretion and loss of gonadal function (Tables 7.4 and 7.5). This applies equally to both male and female animals. The extent of the

Table 7.5. *Some observations on the effect of immunization of female animals with gonadotrophin-releasing hormone (GnRH)*

References	Species	Findings
Arimura *et al.* (1974)[25]	Rat	Suppression of ovulation and gonadotrophin secretion by antiserum to GnRH
Clarke *et al.* (1978)[26]	Sheep	Suppression of LH, FSH and ovarian cyclicity
Fraser & Baker (1978)[27]	Rat	Loss of ovulation and cyclic gonadal function; animals with low antibody titres had low gonadotrophins and hypogonadism
Kawakami & Higuchi (1979)[28]	Rat	Suppression of LH but not FSH after active immunization; passive immunization suppressed both LH and FSH
Okon *et al.* (1980)[29]	Rat	Suppression of ovulation and gonadotrophin secretion; atrophic changes in pituitary, ovaries and uterus (cystic glandular dilatation); animals with low titres showed squamous metaplasia of endometrium
Gledhill *et al.* (1982)[30]	Ferret	Rapid fall in LH, delayed fall in FSH
Fraser (1983)[31]	Monkey	Loss of LH and FSH surges; low E2 and absence of P
Esbenshade & Britt (1985)[32]	Pig	Suppression of LH, FSH and ovarian function; not restored by administration of GnRH or gonadotrophins, but sensitive to GnRH analogue
Short *et al.* (1985)[33]	Wallaby	Administration of anti-GnRH reduced LH and FSH, inhibited follicular development during pregnancy and prevented post-partum oestrus and ovulation
Garza *et al.* (1986)[34]	Horse	Low LH, FSH and P; no increase after ovariectomy
Safir *et al.* (1987)[35]	Horse	Suppression of ovulation in animals with high antibody titres
Ravdin & Jordan (1988)[36]	Rat	Protects against dimethylbenanthracene-induced mammary tumours; suppression of gonadal function
Taya & Sasamoto (1988)[37]	Rat	Administration of anti-GnRH to lactating rats reduced LH and increased FSH

Note:
E2 = oestradiol; P = progesterone; for other abbreviations, see Table 7.4.

inhibition of reproductive activity is related to the level of immunity: animals with low antibody titres may show reduced but not absent pituitary–gonadal activity.[27] Pituitary–gonadal function can be restored in immune animals by administration of GnRH analogues which do not react with anti-GnRH.[13]

In animals, the only adverse effect which has been described as a result of immunity to GnRH is cystic change and squamous metaplasia of the endometrium in rats.[14] In the human, immunity to GnRH would be expected to lead to Type 1 disorders in both females and males (Tables 7.2 and 7.3).

Table 7.6. *Observations on the effect of immunization of monkeys with luteinizing hormone and follicle-stimulating hormone*

References	Species (sex) and immunogen	Findings
Prahalada *et al.* (1975)[39]	Monkey (F) sheep LH	Injection of anti-LH inhibits ovulation and terminates pregnancy
Sundaram *et al.* (1976)[40]	Monkeys (F) beta-LH sheep	Suppression of fertility but no interference with ovulation or regular menstrual cycles
Moudgal *et al.* (1978)[41]	Monkey (F) sheep LH	Injection of anti-LH inhibits ovulation and terminates pregnancy
Murty *et al.* (1979)[42]	Monkey (M) sheep FSH	Injection of anti-FSH suppressed spermatogenesis
Thau *et al.* (1979)[38]	Monkey (F) beta-LH sheep	75% of animals developed antibodies to LH; ovulation and regular menstrual cycles continued but P levels reduced
Wickings & Nieschlag (1980)[43]	Monkey (M) sheep FSH	Suppression of spermatogenesis, returning to normal after some years; serum T and LH normal, and normal ejaculations
Spinola *et al.* (1982)[44]	Monkey (F) sheep beta-LH	Shortened luteal phase and reduced P levels; no change in E2 levels; fertility restored by P treatment
Srinath *et al.* (1983)[45]	Monkey (M) sheep FSH	Suppression of spermatogenesis, returning to normal after some years; serum T and LH normal, and normal ejaculations
Thau *et al.* (1983)[46]	Monkey (F) sheep beta-LH	hCG restores luteal function in immunized animals
Thau *et al.* (1983)[10]	Monkey (F) sheep beta-LH	Immunization includes circulating immune complexes and some arterial changes (intimal thickening)
Thau *et al.* (1986)[47]	Monkey (F) sheep beta-LH	Immunization reduces fertility but does not prevent ovulation or disturb cycle; controlled study showed no increase in atherosclerosis with long-term immunization
Thau *et al.* (1987)[48]	Monkey (F) sheep beta-LH sheep FSH	Long-term immunization did not impair pituitary function (normal response to GnRH; normal pituitary histology)

Note:
E2 = oestradiol-17-beta; P = progesterone; T = testosterone; LH = luteinizing hormone; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotrophin.

Antibodies to LH and FSH

LH

The evidence on the effect of immunization to LH is much less clear cut than that for GnRH. In the rhesus monkey, immunization with LH can reduce

Table 7.7. *Observations on the effect of immunization of animal species other than monkeys with luteinizing hormone*

References	Species (sex) and immunogen	Findings
Spies & Quadri (1967)[49]	Sheep (F) sheep LH	Injection of anti-LH terminates pregnancy
Loewitt & Laurence (1969)[50]	Rat (F) bovine LH	Injection of anti-LH terminates pregnancy
Munshi *et al.* (1972)[51]	Mouse (F) sheep LH	Injection of anti-LH terminates pregnancy
Rao *et al.* (1972)[52]	Hamster (F) LH, FSH	Injection of anti-LH terminates pregnancy
Tandon *et al.* (1984)[53]	Mouse (F) sheep LH or hCG	Injection of anti-LH or anti-hCG terminates pregnancy; counteracted by administration of P
Malecki *et al.* (1987)[54]	Goat (pregnant) sheep LH	No effect on pregnancy or P levels

Note:
For abbreviations, see Table 7.6.

fertility without affecting ovulation.[38] Consequently, it has been proposed that the principle effect is on early pregnancy. A number of studies have shown that passive administration of anti-LH can lead to failure of the corpus luteum with reduced progesterone secretion and loss of a pregnancy (Tables 7.6 and 7.7). This can be corrected by administration of progesterone or stimulation with hCG. Detailed studies on rhesus monkeys following long-term immunization with sheep β-LH have shown no evidence of pituitary dysfunction.[48]

In humans, it would be expected that neutralization of LH would lead to a Type 3 disorder (see Table 7.2). However, if the situation is analogous to that in monkeys, ovulation and normal ovarian function might continue unimpaired.

FSH

The only experimental evidence on the effects of immunity to FSH is in male rhesus monkeys (Table 7.6). Spermatogenesis was suppressed without alteration in LH, testosterone or number of ejaculations.[43] However, spermatogenesis gradually returned over a period of 4 years. With the realization that spermatogenesis can be maintained with testosterone alone, selective FSH suppression is unlikely to be a viable contraceptive for use by men.

In human females immunity to FSH might be expected to lead to a Type 2 disorder (see Table 7.2). In men, the effects are likely to be the same as those seen in monkeys.[43]

Table 7.8. *Observations on the effect of immunization of animals with oestrogens*

References	Species (sex) and immunogen	Findings
Pant & Rawlings (1973)[55]	Sheep (F) E2	Elevation of LH and cessation of oestrus cycle
Kaushansky *et al.* (1977)[56]	Rat (F) E or P	Prolonged oestrus cycle (E and P); failure of pro-oestrus LH rise but normal fertility in all E-immunized animals; no pregnancies in P-immunized animals
Martin *et al.* (1978)[57]	Cow E2	Loss of oestrus cycle and LH surge; no rise of P
Martenz *et al.* (1979)[58]	Sheep (F) E2, or E1, or T	Basal level of LH and frequency of LH pulses increased in all immunized animals; FSH increased in some but not all animals
Wielgosz *et al.* (1980)[59]	Rabbit (F) E2	Increased circulating LH, FSH, T, E2 and P; polycystic ovaries
Nishihara & Takahashi (1983)[60]	Rat (M) E2	Increase in circulating LH and T levels
Samperez *et al.* (1983)[61]	Rat (F) E2	Elevated LH, reduced FSH; polycystic ovaries; loss of cyclicity
Thompson & Honey (1984)[62]	Horse (M) E1	Immunization of pre-pubertal colts enhanced testicular size, sperm production and T levels
Wise & Ferrell (1984)[63]	Heifer E2	Increased weight gain; polycystic ovaries with no corpora lutea
Boland *et al.* (1987)[64]	Sheep (F) E2	Suppression of gonadotrophin-induced ovulation at highest titres, enhanced at lower titres; GnRH produced equal LH in surge
Bigsby *et al.* (1986)[65]	Rabbit (F) E2	Administration of anti-E2 acclerated transport of ova in oviducts and expulsion from uterus
Jenkins *et al.* (1986)[66]	Sheep (M) E2	Administration of anti-E2 produced an increase in T but no effect on LH, FSH or prolactin
Lehar *et al.* (1987)[9]	Mouse (F) E2	One-third of immunized animals developed antinuclear antibodies
Schanbacher *et al.* (1987)[67]	Sheep (M) E2	Increase in LH, FSH, T and testicular weight
St John *et al.* (1987)[68]	Cow E2	Increased lipogenesis
Zelesnik *et al.* (1987)[69]	Monkey (F) E2	Administration of anti-E2 during luteal phase led to increase in LH and FSH
Thompson & Johnson (1987)[70]	Horse (F) E2	No effect on prolactin levels
Monet-Kuntz *et al.* (1988)[71]	Sheep (M) E2	Increase in LH, FSH, T, testicular weight and spermatid production
Sanford (1989)[72]	Sheep (M) E2	Administration of anti-E2 produced an increase in T but no effect on LH

Note:
E1 = oestrone; E2 = oestradiol; P = progesterone; T = testosterone; A4 = androstenedione; LH = luteinizing hormone; FSH = follicle-stimulating hormone.

Antibodies to gonadal steroids

One feature which is common to all animals immunized with gonadal steroids is an increase in endogenous circulating levels of the steroid. This is principally in the form of a bound complex with the antibody and hence is not available to exert biological effects. By contrast, animals immunized with peptide hormones generally have lower circulating levels of the target material.

Oestradiol

Studies on the effects of immunization against oestradiol (E2) have yielded inconsistent results, both between species and between different investigations on the same species (Table 7.8). In some studies, ovarian function was found to be stimulated with development of polycystic ovaries; in others the procedure led to anovulation. Most studies agree that there is an elevation of LH and FSH (due to lack of feedback inhibition). Loss of fertility has been shown in some but not all studies. In the male, fertility may be enhanced by immunization with oestrogen.[60,62]

In the human, neutralization of oestradiol should suppress the midcycle LH surge which is the direct result of the late follicular oestradiol peak. The loss of both ovulation and oestrogen effects would be expected to yield a Type 1 disorder. However, as the animal experiments demonstrate, the effect could be far more complex than this including, paradoxically, an enhancement of ovarian activity.

Progesterone

Passive immunization with antiserum to progesterone (P) causes loss of early pregnancy, presumably due to loss of the normal luteal support to the endometrium. A similar abortifacient effect might be expected in humans. Active immunization of female animals with progesterone appears to promote fertility[73] (Table 7.9).

Testosterone

In the female, neutralization of testosterone (T) leads to a reduction of FSH and a small increase or no change in LH (Table 7.9). Ovulation has been variously reported as being suppressed or increased, the latter especially after immunization with androstenedione. In the male, there is usually increased LH and FSH and suppression of testicular function with loss of libido. However, active immunization of 3-month-old bulls leads to subsequent enhanced development of testicular function. In the adult human male, immunization with testosterone would be expected to lead to an increase in LH together with suppression of testicular function and loss of libido.

Table 7.9. *Observations on the effect of immunization of animals with gonadal steroids other than oestrogens*

References	Species (sex) and immunogen	Findings
Hillier *et al.* (1974)[74]	Rat (F) T	Suppression of ovulation and development of polycystic ovaries
Nieschlag *et al.* (1974)[75]	Rabbit (M) T	Suppression of testicular function; increased LH
Scaramuzzi *et al.* (1977)[76]	Sheep (F) A4	Disruption of ovarian cycle and increased number of ovulations
Armstrong *et al.* (1978)[77]	Rabbit (F) T	Reduced FSH and androgens; normal LH; increased E2 and number of ovulations
Gay & Kerlan (1978)[78]	Rat (M) T	Administration of anti-T produced a rise in LH and FSH
Scaramuzzi *et al.* (1981)[79]	Sheep (F) T	Disruption of ovarian cycle and increased number of ovulations
Cox *et al.* (1982)[80]	Sheep (F) A4 or T or E1	Multiple ovulations and increased lambing rates
Sharpe & Fraser (1983)[81]	Rat (M) T	Increase in LH and FSH; increase in number of LH receptors on Leydig cells
Wise & Schanbacher (1983)[82]	Heifer A4 or E2	Increased ovulation rate in animals immunized to A4
Wang *et al.* (1984)[83]	Mouse (F) P	Injection of anti-P after mating blocks pregnancy in BALB/c mice but not F1 hybrids; effect reversed by administration of P
Rider *et al.* (1985)[84]	Mouse (F) P	Injection of anti-P after mating blocks pregnancy in BALB/c mice; effect reversed by administration of P
Chang *et al.* (1987)[85]	Heifer	Increase in LH secretion and ovarian function; no change in FSH
D'Occhio *et al.* (1987)[86]	Bull calves T or E2	Accelerated growth of testes and enhanced steroid response to gonadotrophin stimulation
Price *et al.* (1987)[87]	Cow T	Anoestrus and polycystic ovaries in some animals, increase in ovulation in others; anoestrus animals showed elevated LH and decreased FSH
Thomas *et al.* (1987)[73]	Sheep (F) P	Increased frequency of LH pulses and ovulation
Thompson *et al.* (1987)[88]	Horse (F) T	Injection of anti-T produced a rise in LH; no effect on FSH, P or ovulation
Rider *et al.* (1986)[89]	Mouse (F) P	Injection of anti-P after mating blocks pregnancy in BALB/c mice; but not F1 hybrids; effect reversed by administration of P
Ellis *et al.* (1988)[90]	Mouse (F) P	Anti-implantation effect of anti-P is specific to high-affinity antibodies
McKinnie *et al.* (1988)[91]	Pig (F) A4	Increased ovulation rate; no effect on survival of embryo

Note:
E1 = oestrone; E2 = oestradid; P = progesterone; T = testosterone; A4 = androstenedione; LH = luteinizing hormone; FSH = follicle-stimulating hormone.

Antibodies to other reproductive antigens

Inhibin

In animals, immunization with inhibin appears to promote fertility in both sexes (Table 7.10). It is therefore unlikely to be a candidate for a contraceptive vaccine in humans.

Porcine zona pellucida

Initially, it was assumed that the antifertility effect of immunization to heterologous zona pellucida was due to inhibition of sperm penetration of the zona matrix surrounding the ovum.[92] However, it is now apparent that the primary effect is a failure of follicular growth (Table 7.10). This is accompanied by an increase in LH and FSH. The detailed studies of Skinner and colleagues[101] have characterized some of the specific proteins involved in this reaction.

Gamete-specific antigens

There is increasing evidence that the development of immunity against gamete (spermatozoon and oocyte) antigens is a feasible objective. Approximately 5% of men and women attending infertility clinics show signs of immunity against sperm-specific antigens without any associated morbidity other than long-term infertility and a reduction in cumulative pregnancy rates. Corticosteroid immunosuppression reduces antibody titres in these patients and increases the fertility rates. Seventy percent of men sterilized by vasectomy develop antisperm antibodies, the presence of which significantly reduces the chances of returned fertility after vasovasostomy. The mechanisms by which such antibodies depress fertility include complement-mediated immobilization of the spermatozoa, IgA-mediated disruption of cervical mucus penetration, and inhibition of the recognition and membrane fusion events comprising fertilization.

The main process affected by antisperm antibodies appears to be the binding of spermatozoa to the zona pellucida[111] – the main cell recognition event in fertilization. A monoclonal antibody directed against a guinea-pig sperm antigen, PH 20, has been shown to be effective in disrupting fertilization both *in vitro* and *in vivo*.[112] Active immunization with this material has an antifertility effect in both male and female guinea-pigs, with the fertility of the animals returning, progressively, over a period of 15 months.

It appears that a vaccine against sperm-specific antigens could control both male and female fertility and that the effects should be fully reversible. The human spermatozoon also possesses surface antigens but these bear little relationship to the guinea-pig PH 20.

Table 7.10. *Observations on the effect of immunization of animals with various reproductive antigens*

References	Species (sex) and immunogen	Findings
Gwatkin *et al.* (1977)[92]	Mouse (F) hamster ZP	Inhibition of fertility
Lustig *et al.* (1978)[93]	Rat (M) STBM	Passive immunization produced testicular damage
Tung & Goldberg (1978)[94]	Mouse (F) sperm	Inhibition of fertility
Goldberg *et al.* (1978)[95]	Mouse (M and F) LDH	Inhibition of fertility
Trounson *et al.* (1980)[96]	Human (F) PZP	Inhibition of sperm binding and fertilization *in vitro*
Ohlson *et al.* (1981)[97]	Sheep (F) bovine prolactin	No change in LH levels
Wood *et al.* (1981)[98]	Rabbit (F) PZP	No ovulation in response to hCG
Sacco *et al.* (1983)[99]	Monkey (F) PZP	Suppression of ovarian function and fertility, gradually returning to normal after 10–15 months
Henderson *et al.* (1984)[100]	Sheep (F) bovine inhibin	Increase in ovulation rate
Skinner *et al.* (1984)[101]	Rabbit (F) PZP	Inhibition of follicle growth; increased levels of LH and FSH; failure to form corpus luteum in response to hCG
Mahi-Brown *et al.* (1985)[102]	Dog (F) PZP	Abnormal oestrus cycles with persistence of high levels of E2 and no rise in P
Cummins *et al.* (1986)[103]	Sheep (F) bovine inhibin	Increase in ovulation rate
Al-Obaidi *et al.* (1987)[104]	Sheep (M and F) bovine inhibin	Immunization of young ewes advances puberty and increases ovulation rate; in rams there was an increase in testis size, sperm count, and plasma FSH
Sacco *et al.* (1987)[105]	Monkey (F) PZP	Suppression of ovarian function and fertility, gradually returning to normal after 10–15 months
Findlay *et al.* (1989)[106]	Sheep (F) bovine inhibin alpha subunit	Increase in ovulation rate; increase in circulating FSH during breeding season
Findlay *et al.* (1989)[107]	Sheep (F) bovine inhibin alpha subunit	Reduction in fertility
Mann *et al.* (1989)[108]	Sheep (F) porcine inhibin alpha subunit	Injection of anti-inhibin causes increase in FSH
Rivier & Vale (1989)[109]	Rat (F) alpha subunit	Injection of anti-inhibin increases FSH levels but not LH; increase in ovulation rate
Wrathall *et al.* (1990)[110]	Sheep (F) bovine inhibin alpha subunit	Increase in ovulation and circulating FSH; increase in FSH but not LH with injection of anti-inhibin

Note:
PZP = porcine zona pellicuda; STBM = seminiferous tubule basement membrane; LDH = lactate dehydrogenase; LH = luteinizing hormone; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotrophin; P = progesterone; E2 = oestradiol.

Table 7.11. *Observations on the effect of immunization of animals with early pregnancy factors*

References	Species (sex) and immunogen	Findings
Stevens *et al.* (1971)[113]	Baboon (F) hPL	Injection of anti-hPL caused abortion in some animals
Yamini & Chard (1972)[114]	Rat (F) hPL	Injection of anti-hPL terminates pregnancy (probably due to antilymphocyte properties)
Gusdon (1972)[115]	Rat (F) hPL	Injection of rabbit anti-hPL terminates pregnancy, followed by long-term infertility
Bohn & Weinmann (1974)[116]	Monkey (F) SP1	Injection of anti-SP1 terminates pregnancy
Gusdon *et al.* (1974)[117]	Rat (F) hPL	Inhibition of fertility; prolongation of oestrus cycles
Gusdon *et al.* (1976)[118]	Monkey (F) hPL	Inhibition of fertility in some but not all animals
Ramakrishnan *et al.* (1978)[119]	Monkey (F) beta hCG	No rise in P after injection of hCG; normal response to hLH

Note:
SP1 = Schwangerschaftsprotein 1; hPL = human prolactin; hCG = human chorionic gonadotrophin; P = progesterone; hLH = human luteinizing hormone.

Antibodies to early pregnancy factors

Administration of antibody to LH or hCG can terminate pregnancy in a variety of animals (Tables 7.7 and 7.11). The effect is probably an abrogation of the gonadotrophin-induced rescue of the corpus luteum, since the effect of the antibodies can be counteracted by administration of progesterone.

Monitoring of women immunized with hCG

It is likely that most large-scale experimental work on antifertility vaccines will, for the foreseeable future, focus on β-hCG or fragments thereof (Table 7.12). Monitoring of the women will obviously include specific tests for the presence of antibodies to the immunogen, and tests for various potential non-specific immunological effects as detailed elsewhere (see Chapters 6 and 9). The main tests for the potential endocrine effects will include the following.

1. In all women a detailed menstrual history for a period of at least 6 months after the last injection of vaccine, or longer if significant antibody levels persist. This should note the onset and duration of menses, a subjective view of amount of blood loss, and a note of associated symptoms (pain, etc).
2. More intensive investigation in at least three of the first six cycles. Objective evidence should be sought for the presence or absence of

Table 7.12. *The effect of immunization of humans with various human chorionic gonadotrophin preparations*

References	Immunogen	Findings
Stevens & Crystle (1973)[120]	Diazo-human hCG (intact	Subjects developed antibodies; reduced levels of LH; ovulation ceased in some but not all women
Talwar *et al.* (1978)[121]	Beta-hCG coupled to tetanus toxoid	Subjects developed antibodies; no effect on ovulation
Nash *et al.* (1980)[122]	Beta-hCG coupled to tetanus toxoid	Antibodies produced in 14 of 15 women; no effect on menstrual or other endocrine functions
Jones *et al.* (1988)[123]	Beta-hCG–C terminal peptide coupled to diphtheria toxoid	Thirty subjects; no important adverse reactions; contraceptive levels of antibodies in all subjects for 6 months

Note:
hCG = human chorionic gonadotrophin.

ovulation (serum progesterone on day 21 of the cycle; ultrasound evaluation of ovaries if available).

In addition to the investigations set out in (1) and (2) above, it is highly desirable that some women should be more intensively investigated, as follows.

3. In a group of at least 12 women, blood samples would be collected on days 18, 21, 24 and 28 of three cycles and assayed for progesterone, PP14 and SP1. Measurement of PP14 and SP-1 might provide evidence of 'occult' pregnancy in women having antibodies to hCG.
4. In a subgroup of women (probably 10–20), daily evaluation of urine LH, serum LH, FSH, prolactin, oestradiol and progesterone throughout one or more cycles.

If an amenorrhoeic state occurs in some women, then the subjects should be followed up regularly over a period of 1–5 years to monitor post-menopausal changes (blood chemistry, coagulation factors, bone scans).

Conclusion

A contraceptive vaccine based on one of the hormones or other antigens involved in the process of reproduction is an attractive theoretical possibility. This concept is reinforced by numerous studies in animals, and a very few in humans, demonstrating the efficacy of the approach. The obvious drawback to the clinical use of many of these vaccines in the human is the side-effects presented as Table 7.13. On the basis of the animal evidence presented in this,

Table 7.13. *A summary of the types of disorder which might arise from immunization of humans with reproductive hormones and other antigens. The numbers refer to the clinical conditions listed in Tables 7.2 and 7.3*

Immunogen	Female	Male
GnRH	1	1
LH	3	3
FSH	2	4
E	1	?
P	3	?
T	?	3
Inhibin	3	?
PZP	?	—
Sperm	—	?
hCG	?	?
hPL	?	?
SP1	?	?

Note:
For abbreviations, see other tables in this chapter.

all of the hormones of the hypothalamic–pituitary–gonadal axis would be excluded. Though some of the side-effects are mild and easily corrected, the need for routine steroid supplementation would negate the benefit of a vaccine whose main potential advantage is ease of use. The only other candidates for a contraceptive vaccine are the antigens specific for early pregnancy, notably hCG, and some of the gamete-specific antigens on the oocyte or spermatozoon. Even with vaccines developed from gamete antigens it is not possible to guarantee that the effects of the vaccine will be restricted to the gametes and will not simultaneously involve gonadal endocrine function. At the present time the only approach which offers both theoretical and practical evidence of safety and efficacy is a vaccine based on the hormone-specific region of β-hCG and this will probably continue to be the only significant candidate for an antifertility vaccine for the foreseeable future.

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8 Immunological hazards associated with human immunization with self or self-like antigens

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Introduction

The immune response is designed to respond to all foreign antigens. A number of mechanisms are in place to avoid responses to constituents of the host's own body. They include deletion of self-reactive precursor cells in the thymus, partial cell deletion and active suppression. The failure of these mechanisms leads to autoimmunity. Whether or not an autoimmune response leads to autoimmune disease depends upon the accessibility of the antigen and the quality of the response. Antigens of the reproductive tract can induce autoimmunity which may entail pathological consequences. A systematic assessment of subjects deliberately immunized with antigens of the reproductive tract begins with an autoantibody screen followed by definition of the corresponding antigens. The critical decision is whether the response produces pathological changes. A number of in-vitro tests can be applied to predict the immunotoxic effects of autoantibodies and of self-directed, cell-mediated autoimmune responses.

Mechanisms of self/non-self discrimination

The induction of the immune response

The development of an immune response results from the interactions of several populations of mononuclear cells and their soluble products, the interleukins and cytokines. The three major populations are antigen-presenting cells (APC), T lymphocytes (T cells), and B lymphocytes (B cells).

The primary role of the APC is to capture protein antigen, to ingest and digest it, and to present fragments of the antigen as oligopeptides in conjunction with the products of the class II major histocompatibility complex (MHC II) at the cell surface (see Chapter 4).

Helper/inducer T cells are endowed with the ability to recognize these antigen fragments if presented with syngeneic MHC II by an APC (see Chapter 4). Following interaction with appropriately presented antigen, the T cell proliferates to produce a clone and to interact with other T cells and with B cells in order to instigate an immune response. Effector T cells produce

lymphokines such as gamma-interferon (to stimulate macrophages) or lymphotoxins (or tumour necrosis factors) to injure target cells. Additionally, cytotoxic T cells are generated and act upon susceptible targets expressing the requisite antigenic determinant in conjunction with class I MHC products. T cells also interact with B cells to assist in the differentiation of the B cell into plasma cells capable of secreting antibody. In addition to inducing these effector immune responses, T cells may regulate or suppress them.

B cells have the unique capability of producing and secreting antibody. In most cases, they require the help of T cells directly and, through the elaboration of particular interleukins, the B cell-stimulating factors. In the case of T cell-independent antigens, the B cells interact directly with APCs and differentiate into antibody-producing cells. B cells are major antigen-presenting cells during the later stages of a primary immune response and during secondary responses when the population of antigen-specific B cells has expanded.[1]

During the earlier stages of the immune response, the major APCs are probably MHC II-bearing cells of the macrophage–monocyte series, including macrophages, dendritic cells of the blood and lymphoid organs, astrocytes of the central nervous system and Langerhans cells of the skin. A number of other cells have been shown to express class II MHC antigens following stimulation by lymphokines, including epithelial and endothelial cells. Under certain circumstances, it has been claimed that such cells may act as APCs.[2]

Discrimination between self and non-self

The immunological system provides sufficient diversity to respond to all foreign antigens. It is, then, particularly critical to identify and understand the mechanisms that avoid immune responses to antigens normally present in the body of the host itself.

At the level of B cells

The simplest hypothesis suggests that the cell populations required to generate an immune response are deleted or silenced during embryonic development. There is, however, strong evidence that B cells reactive with many self-antigens are present in normal animals (see Chapter 1). The earliest evidence was based on demonstration of naturally occurring autoantibodies to a great variety of intracellular and extracellular constituents. Later it was found that many of the abnormal monoclonal antibody products of malignant B cells, myelomas or plasmacytomas, are autoantibodies. Finally, direct evidence has come from hybridomas produced using B cells from normal animals. A large proportion of the monoclonal antibodies derived from such hybridomas have autoantibody activity directed to both intracellular and cell surface antigens.[3,4] It seems most likely, therefore, that clonal deletion or anergy does not generally apply to B cells except for the most prominent cell surface alloantigens such as products of the MHC or major blood groups.

At the level of APCs

Because of the essential role of the APC, several investigations have recently been carried out in which the ability of APCs to present self antigens has been compared to presentation of foreign antigens. At least three different antigens have been studied in some detail – haemoglobin, lysozyme and insulin. In each case, APCs are able to present the autologous form of antigen as readily as the xenogeneic antigen.[5] These findings suggest that discrimination between self and non-self is not made at the level of antigen presentation, as judged by the production of specific autoantibodies.

At the level of T cells

The role of T cells in self-tolerance is still a subject of much investigation. A deletion mechanism eliminates the precursors of T cells reacting with the most prominent cell surface alloantigens.[6,7] The development and 'education' of T cells in the thymus entail further selective processes, apparently favouring T cells with affinity for self-MHC joined with a foreign antigenic determinant but eliminating T cells with high-affinity receptors for self-MHC plus self-antigens. These processes mainly occur during the migration of T cells from the thymic cortex to the medulla.[8] There is, on the other hand, considerable evidence suggesting that T cells reactive with other self-antigens are not entirely deleted during their thymic differentiation. T cells responsive to mouse thyroglobulin, for example, can be found in normal mice.[9]

It has recently been proposed that the important parameter with respect to self-reactive T cells is their affinity for self-peptide plus self-MHC.[10] Higher affinity T cells are more likely to be deleted in the thymus by the selection mechanism described, whereas T cells with lower affinity for MHC or for self-peptides escape. They seed peripheral lymphoid organs where they can be triggered by a sufficiently potent antigenic signal.

Active suppression

Based on current evidence that normal animals possess B and T cells that are capable of recognizing many self-antigens, mechanisms involving active suppression have been invoked to explain the absence of pathologically significant autoimmune responses. A number of investigations point to the presence of antigen-specific suppressor T cells[11] which can be readily elicited and demonstrated if soluble antigen is given by an intravenous route without an adjuvant. Their production, then, may depend mainly upon the details of antigen presentation and be influenced by the genetic constitution of the host. There has, in addition, been recent evidence showing that anti-idiotypic responses can obviate autoimmune reactions.[12] Anti-idiotypic responses are initiated by the unique antigen-combining portions of the antibody molecule or of the T-cell receptor and involve the production of antibodies or T cells specific for the idiotypic or clonotypic determinants.

Multiple mechanisms

The preponderance of current evidence, therefore, suggests that several mechanisms of preventing autoimmune responses obtain in normal animals.

(a) Clonal deletion of B cells effectively prevents anti-self responses to MHC and MHC-like determinants including the major blood group antigens.
(b) In the case of other self-antigens, clonal deletion of B cells either does not occur or is incomplete, but the deletion of more self-reactive T cells prevents the generation of autoimmunity.
(c) Antigen-specific regulatory T cells, or anti-idiotypic antibodies or T cells, actively suppress the development of autoimmunity.

Targets of autoimmunity

Antigen location

Sequestered antigens

Based on the considerations raised above, the induction of autoimmunity depends in the first place upon the location of the self-antigen. A few antigens may be sufficiently sequestered from the immune system that tolerance to them never develops. Classical examples cited are the lens of the eye and the sperm of the testes. Lens antibodies can be found following injury to the lens.[13] Antibodies to sperm occur in animals after ligation of the vas deferens preventing the normal exit of sperm from the body.[14] Following vasectomy of humans, for example, about 80% of men produce antibodies to their sperm. Antibodies rarely reach sufficient concentration in sequestered sites to produce pathological changes.

Circulating antigens

In the case of constituents accessible to the circulation, concentration of antigen seems to be a major parameter. Proteins present in the bloodstream in relatively small amounts, such as thyroglobulin, induce relatively poor self-tolerance. If introduced with a potent adjuvant, such as Freund's complete adjuvant, most animals can be induced to respond to their own thyroglobulin.[15] The extent of response is regulated by the host's MHC II genes.[16] In fact, low levels of circulating autoantibodies to thyroglobulin are frequent in normal individuals and their frequency mounts with age.[17] This characteristic rise in prevalence, which is seen with many other autoantibodies, may result from:

(a) contact with environmental antigens having shared determinants;
(b) an accumulation of somatic mutations; or,
(c) a loss of active suppression due to thymic involution during ageing.

A tenfold increase in the normal circulating levels of thyroglobulin can be

induced by thyroid-stimulating hormone or thyrotrophin-releasing hormone. It will depress the ability of an animal to produce an autoimmune response, illustrating the importance of the concentration of antigen in the circulation in maintaining tolerance to self-antigens.[18] The reverse, however, does not hold, since reducing the concentration of antigen does not seem to increase the risk of autoimmunization.

With respect to homogeneous plasma proteins circulating in high concentrations, rarely, if ever, do they induce an autoimmune response; the absence of reports of autoantibodies to serum albumin is a pertinent example. In contrast, autoantibodies to immunoglobulins are found under special conditions. Rheumatoid factors sometimes appear to determinants of the Fc portion of immunoglobulin which are allosterically altered by antigen–antibody interaction so that they assume the appearance of alloantigen.[19] Anti-idiotypic antibodies are easily produced to the variable portions of the immunoglobulin molecule, since a particular idiotype is present in relatively low amounts, too low to induce and maintain self-tolerance.[20]

Internal constituents of cells

Autoantibodies to many internal constituents of cells are frequent in completely normal individuals; for example, autoantibodies to cytoskeletal components, such as vimentin, fibrillin, actin and myosin, are almost universally present in normal human sera.[3] Low levels of antinuclear antibodies are also present normally.[21] Under usual circumstances these autoantibodies circulate in low titres and have relatively low affinities. Since they increase in titre and affinity in certain diseases, one can use elevated titres of antinuclear antibodies as an aid in the diagnosis of systemic lupus erythematosus (SLE); of antimitochondrial antibodies in primary biliary cirrhosis; and of antibodies to actin in chronic active hepatitis.[22] In each instance, the antibody is thought of as a marker of disease rather than the direct cause. Yet sometimes the intracellular antigen is released in sufficient quantity to combine with the antibodies to produce circulating immune complexes which can be pathogenic, as seen in lupus nephritis.

Thus expression of autoimmunity evidenced by the presence of autoantibodies to both internal and circulating antigens is frequent. These autoimmune responses are generally unaccompanied by overt disease although antibodies to circulating hormones and enzymes may impede and interfere with physiological function. It is impressive, though, how rarely a functional effect can be documented. Perhaps high-affinity autoantibodies are generally not formed to the conserved hormonogenic or catalytic portions of the molecule, which are shared by the antibody-forming host itself.

Cell-surface antigens

In dealing with cell-surface antigens, antibodies alone or in conjunction with complement may play a direct pathogenic role. In patients with autoimmune

forms of haemolytic anaemia, leukopenia or thrombocytopenia, autoimmune responses occur to circulating erythrocytes, leukocytes and platelets, respectively. For example, red blood cells coated with autoantibody at cold temperatures are lysed after warming through the agency of complement in cases of paroxysmal cold haemoglobinaemia. In the warm antibody forms of haemolytic anaemias, autoantibody-coated red blood cells are taken up by phagocytic monocytes of the spleen and liver. Autoantibodies to cell-surface receptors can block receptor function, as seen in patients with myasthenia gravis, who have autoantibodies to the acetylcholine receptor. In Graves' disease, some patients produce autoantibodies that increase the metabolic activity of thyroid epithelial cells by acting on the thyrotrophin receptors, resulting in hyperthyroidism.

Internal cell antigens

In the case of antigens located within living tissue cells, antibodies are unable to reach their target in significant amounts, so that antibody-mediated disease occurs only when the antigen is spilled into the bloodstream. Autoimmune lesions to antigens in cells of solid tissues probably occur only when cell-mediated immunity is aimed at a tissue antigen. Lymphocytes actively leave the vascular compartment and enter intercellular spaces. Like antibodies, cytotoxic T cells probably do not penetrate viable intact target cells and can produce damage only if peptides from the requisite antigen are expressed at the cell surface in conjunction with MHC I products. Once they recognize the tissue antigen and localize in a particular tissue site, however, T cells initiate an inflammatory response, producing mediators of tissue damage as well as lymphokines that attract additional mononuclear cells. This inflammatory response provides an opportunity for APCs, antigen-specific helper and cytotoxic T cells as well as for antigen-specific B cells to accumulate in tissue sites and may, thereby, inaugurate a vicious cycle of autosensitization and autoaggression. The appearance of cell-mediated immunity, then, appears to be the critical step for the development of autoimmune lesions of most solid tissues. The final picture of cell damage, however, may well depend upon a composite of antibody-dependent complement-mediated lysis, antigen-specific cytoxic T-cell injury, antibody-dependent cell-mediated damage, as well as effects of natural killer (NK) cells and activated macrophages.

Antigens of the reproductive tract

Hormones

The obvious need for new fertility-regulating methods has led to the development of vaccines against components of the reproductive tract. The most advanced fertility-regulating vaccines are the ones against the placental hormone, human chorionic gonadotrophin (hCG). A variety of different antigen formulations has been developed, each of which includes potential endocrinological and immunological hazards and drawbacks. In this section,

Table 8.1. *Amino acid sequence similarities (percentage homology)*

β-hLH/β-oLH	68%
β-hCG/β-oLH	61%
β-hCG/β-hLH	85%
human α/ovine α	74%

Note:
LH = luteinizing hormone; CG = chorionic gonadotrophin; o = ovine; h = human.

therefore, emphasis will be placed on immunological and biological similarities of hormonal vaccine components with related molecules of the endocrine system and possible immunopathological consequences resulting from these relationships.

HCG: biological versus immunological specificity

HCG belongs to an evolutionary and biochemically closely related glycoprotein family comprising luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH) and hCG. All members are heterodimers consisting of an α- and a β-subunit. Within a given species the α-chain is encoded by a single gene[23,24] and, therefore, not distinguishable by immunological means. On the other hand, β-subunits are encoded separately, are different from hormone to hormone, and confer immunological and biological specificity. There is one exception to this rule: β-hLH and β-hCG have probably evolved from a common ancestral gene by a read-through event resulting in an additional C-terminal peptide (CTP) on β-hCG.[25] Amino acid sequences and the gene structures of both subunits have already been established in various species.[26,27]

Until recently, a concept by J. Vaitukaitis[28,29] was generally accepted and frequently cited in the literature.[30,31] It proposed that α-subunits are immunologically species specific despite high interspecies amino acid sequence homologies (Table 8.1). This idea was probably the basis for the development of vaccine formulations which use α-oLH subunits annealed to β-hCG (Table 8.2) in order to endow the β-chain with a more holo-hormone like molecular configuration without risking elicitation of unwanted antibodies which cross-react with the other glycoprotein hormones. However, the above-mentioned concept was disproven by the fact that three out of six different epitopes on human glycoprotein hormone α-chains, which were defined by a panel of over 140 monoclonal antibodies, are shared by the α-subunit of various species.[32,33]

With the exception of β-hLH/β-hCG, human β-chain amino acid sequence dissimilarities are, in general, great enough to evoke hormone-specific immune responses. Looking at the immunological makeup of β-hCG in more detail,

Table 8.2. *Immunogens used in anti-hCG vaccines*

World Health Organization
β-hCG–CTP–diphtheria toxoid (DT)[a]
Indian National Institute of Immunology
β-hCG–tetanus toxoid (TT)[a]
β-hCG:α-oLH–TT and β-hCG:α-oLH–cholera toxoid chain B (CHB)[a]
β-hCG–TT, β-oLH–TT, β-hCG–CHB and β-oLH–CHB[a]
Population Council
β-hCG–TT[a]
β-oLH
β-hCG–TT and β-oLH

Note:
[a] Preparations evaluated in Phase 1 clinical trials.

different epitope qualities become visible. Epitopes can be hormone-specific, located either on the β-hCG core[34] despite an approximately 85% sequence homology between β-hCG and β-hLH, or on the β-hCG–CTP, predisposing them for use in fertility-regulating vaccines. All other antigenic determinants are present on hLH or even on the evolutionary more distant hTSH.[35] As the biological specificity of a glycoprotein hormone is dependent on its β-subunit[36] but independent of the species of orgin, it is not surprising that many of the non-hCG-specific determinants are shared between species.[34,37,38] Vaccines containing β-subunits of non-human species make use of this interspecies sharing of determinants; on the other hand, the use of such formulations carries the risk of eliciting intraspecies cross-reacting antibodies.

Composition of vaccines

Vaccine compositions have been reviewed recently by V.C. Stevens.[31] Three institutions, namely, the Indian National Institute of Immunology, the Population Council, and the World Health Organization, have developed immunogens consisting of various combinations of glycoprotein hormone subunits or fragments and different carriers as well as different adjuvants (Table 8.2).

Mechanisms of vaccine action

After ovulation and fertilization of the ovum, the implanting blastocyst will secrete hCG and free, non-assembled α-hCG and β-hCG subunits.[39] The stage at which hCG secretion first occurs, i.e. before or after implantation, is still a matter of debate, although it has been reported recently that mRNA for β-hCG has been detected in human embryos at the eight-cell stage.[40,41] Immunological prevention of pregnancy might be possible by two mecha-

nisms: either by antibodies or by cell-mediated cytotoxicity. Neutralization of gonadotrophic hormones by means of polyclonal antisera resulting in resorption of the conceptus is certainly possible, as previously shown in the rat system.[42] As polyclonal antisera as well as monoclonal antibodies can specifically interfere with hormone–receptor interaction *in vitro*, the decline of corpus luteum function, i.e. a dramatic drop in progesterone levels *in vivo*, might be due to the same mechanism. Neutralizing antibodies either recognize or alter the molecular configuration of the biologically active centre of the hormone or sterically inhibit receptor recognition including hindrance by equal charge. As the receptor-binding and activation sites should be very similar on hLH and hCG, they should not be used as vaccine immunogens; this is one advantage of the WHO-supported hCG-unique CTP formulation. Until now, it has not been demonstrated whether antibodies against the CTP inhibit hormone–receptor interaction directly or exhibit 'transneutralizing' capacity turning hCG into an antagonist or partial antagonist which does bind to the ovarian receptor but without transfer of the hormonal message, or only at a reduced rate.

Potential hazards

The use of non-hCG-specific hormonal antigens for immunization obviously carries risks of undesired side-effects on the endocrine system, the female reproductive tract, the thyroid and the kidney, which possibly excludes them from widespread usage.[43]

Some determinants on β-hCG which are shared by LH and CG of various species[34,37,38] are still accessible on preformed hormone–receptor complexes;[37,44] the question whether this also applies to the CTP remains to be answered. If this is the case, a target organ attack via epitopes on cell surface-bound hormones, either by antibodies plus complement or by cell-mediated cytotoxicity, would not be restricted to β-hCG or β-oLH vaccines but is possible in all cases where β-LH, β-hCG or peptides thereof are used for immunization.

As shown in rats, the use of antibodies for hormone neutralization can lead to the formation and subsequent deposition of immune complexes in the kidney, but this might only apply to passive immunizations.[45]

Perhaps the most important risk of non-CTP vaccines is the potential damage by antibody or cytotoxic lymphocytes to LH-producing cells in the pituitary or to LH-target cells in the gonads and other tissues. In addition, there is a risk of immunological interference with TSH causing primary and secondary hypothyroidism. As has been shown recently with the aid of monoclonal antibodies, antigenic determinants are not only shared between β-hCG and β-hLH, and between the α-chains of ovine and human glycoprotein hormones,[32,33] but, surprisingly, even between hCG and β-hTSH,[35] and β-hCG and α-hCG.[46] Moreover, antibodies against such epitopes can neutralize hormone receptor-binding ability *in vitro*. Additionally, a shared

β-epitope is exposed on receptor-bound hormone (Berger *et al.*, unpublished observation), thus theoretically providing the basis for an autoimmune attack.

Further drawbacks

1. *Free α and β-subunit function in vivo.*
   All of the potential vaccines are designed in such a way that they will elicit an immunological response not only towards glycoprotein holohormones but also against the free α and β-chains, respectively. So far, no data are available on possible in-vivo functions of these naturally occurring non-assembled subunits; are they just by-products of endocrine secretion without function or is there a role for them as regulatory molecules in feedback mechanisms in the pituitary–gonadal axis?
2. *Recognition of all hormone variants.*
   Hormonal molecular heterogeneity has its basis in different degrees of sialyation, sulfation, phosphorylation and, rarely, in amino acid sequence variations. Concerning efficacy of the vaccines, the question arises whether all of the post-translational modified variants of hCG[47] will be recognized and neutralized by the antibodies. Although recent studies with monoclonal antibodies derived from inbred mice (Balb/c), which are directed against more than a dozen different epitopes, suggest that any major component of hCG can be detected in Western blots by each antibody,[48] this might not be the case within a normal human outbred population. Furthermore, it is of interest whether individuals of different genotypes will respond equally well to, and consequently be protected from pregnancy by, the synthetic peptide vaccine.
3. *Stimulating antibodies.*
   Immunization with glycoprotein hormone subunits or peptides will result in the production of both neutralizing and non-neutralizing antibody populations. A third and mostly neglected kind of antibodies are those which do not neutralize but even enhance hCG bioactivity.[49] Thus a delicate balance between non-neutralizing, neutralizing and stimulating antibodies may exist *in vivo* which is certainly not reflected by simple measurements of anti-hCG titres. The disturbance of this balance might therefore lead to the opposite of what is desired.
4. *Epitope sharing, female cycle and efficacy.*
   β-hCG-core-specific epitopes exist but antibodies against them are rare;[34] β-hCG–CTP determinants are also known to be of extremely low immunogenicity even in their native form in the intact β-hCG molecule. Therefore, with regard to vaccines using β-oLH and/or β-hCG, neutralization seems to be mediated via cross-reacting anti-

bodies against shared β-chain epitopes. In some subjects taking part in Phase 1 clinical trials of whole β-hCG vaccines, antibodies have been elicited which cross-react with hLH by 100% or more. However, no evidence has been obtained of menstrual cycle disruption or shortening in these individuals (Talwar *et al.*, unpublished information). The question then arises: why isn't the bioactivity of hLH impaired or even completely abolished in these subjects, when their immune responses are considered adequate to neutralize hCG? If this is considered to be due to differences in antibody affinities for the two hormones, it should be expected that the desired high anti-hCG-titres might lead to amenorrhoea.

5. *Reversibility and internal booster effect.*
A prerequisite for a fertility-regulating vaccine is its reversibility – a requirement which is probably met by the β-hCG–CTP design – the drop of protective antibody titres with time being an intended and desired phenomenon.

The perpetuation of immune responses against β-oLH/β-hCG vaccine formulations by internal hLH boost in the absence of carriers (tetanus toxoid, diphtheria toxoid) is not considered likely, as the nanomolar antigen concentrations that would be involved usually fall within the low zone tolerance of the immune system. However, similar situations may also be confronted under other circumstances, e.g. during infections, and the possibility should be borne in mind.

Sperm antigens

Sperm were the first cells to which an autoimmune response was demonstrated. In the original experiments of Metchnikoff, it was reported that autoantibodies acting upon sperm in the test tube could be induced in guinea-pigs by immunization with sperm suspensions.[50] Although autoantibodies could be found in serum, no damage was evident in the testes of the experimentally immunized guinea-pig. If, however, the sperm were removed and mixed in the test tube with normal guinea-pig serum, immobilization and lysis of the spermatozoa took place. These experiments provided evidence that autoantibody is able to penetrate the blood–testis barrier and reach the sperm in the testis, but complement is not present in sufficient quantities to produce sperm damage.

Antibodies to sperm are found in some infertile men.[51] Clinical investigators sometimes treat infertile men with immunosuppresive agents to increase the possibility of successful conception.[52] A correlation between the decreasing titre of antibodies and increased fertility has been reported. Men who have had vasectomies may wish to have their vasectomy reversed by surgery, restoring the presence of sperm in the ejaculate. Some, but not all, such men show restoration of fertility. According to recent reports, men who are still infertile show a relationship between the presence of circulating sperm

antibodies and infertility. There is, then, circumstantial evidence that autoantibodies to sperm interfere with fertility.

Some infertile women produce alloantibodies to human sperm, including sperm from their sexual partners.[53] Successful conception in some infertile couples treated with immunosuppressive agents has been correlated with a fall in antibody titres in these women, indicating another possible connection between circulating sperm antibodies and infertility.[54] A better correlation has been reported between the levels of secretory antibody in vaginal washings and infertility.[55] Consequently, an in-vitro test of mucus penetration by sperm has been proposed as an indicator of female infertility due to alloantibody production to sperm.[56]

The antigens of sperm responsible for immunological infertility have not yet been clearly defined. Studies by immunofluorescence suggest that the major reaction of both male and female sera occurs with the acrosome. At least two acrosomal enzymes have been studied in some detail. Acrosin, a proteolytic enzyme required for penetration of the zona pellucida, is antigenic, but active immunization with acrosin does not seem to produce a reduction in fertility. Hyaluronidase, another acrosomal enzyme, has the ability to disperse cells of the cumulus oophorus. Some investigators have reported a reduction in fertility of female rabbits immunized with this enzyme, but these findings have not yet been confirmed.

Lactic dehydrogenase C4

The best characterized sperm-specific antigen is lactic dehydrogenase X (now termed LDH C4). This enzyme has been isolated from mouse testes and characterized; it has an molecular weight of 140 kDa.[57] Active immunization with murine LDH C4 causes a reduction in fertility of female rabbits, mice and baboons. Instillation of the enzyme into the reproductive tract of female mice produces local immunity with IgA production and a marked fertility reduction.[58] Goldberg and his colleagues[59] have identified the antibody-binding sites on the LDH C4 molecule and have prepared synthetic peptides to replace the intact enzyme. One such synthetic peptide was conjugated to diphtheria toxoid, and used to immunize baboons. The animals produced antibody that bound to native LDH C4 and showed a 70% reduction in fertility compared with non-immunized controls.

Zona pellucida

The zona pellucida comprises a glycoprotein coating that surrounds the ovum and plays an important role in sperm/egg interactions. It has been the object of a number of investigations which showed that animals immunized with zona pellucida are relatively infertile.[60] High-titred antibodies were produced in a number of species by immunization with porcine zona pellucida mixed with complete Freund's adjuvant, and pointed to the presence of a cross-reactive antigen common to the zonae of several species.[61] This form of immunization

seems to produce diminished ovulation as well as a steroid hormone imbalance. The key question is whether it is possible to interrupt sperm/egg interaction without altering the ovarian environment or hormonal balance. To answer this question, further studies of the fine structure of the zona pellucida antigens are required.

Mechanisms of immunopathology

The following discussion of immunological hazards will focus mainly on the possible immunopathological consequences of an hCG subunit vaccine, but will also describe other pathological consequences of autoimmunization.

Autoimmune lesions due to antibody-mediated immunity

Immune complex diseases

Antigen–antibody complexes are involved in the pathogenesis of a large variety of human and animal diseases. Their chronic presence in the bloodstream signals inefficient removal of antigen and/or continued seeding of antigen. These conditions may obtain following immunization with an antigen of the host. Autologous antigens often participate in immune complex formation. For example, DNA may be released into the bloodstream following chronic graft versus host reactions in F1 hybrid mice given parental spleen cells.[62] The first effect of the liberated DNA is to stimulate the formation of circulating anti-DNA antibodies. This is followed, a few days later, by the appearance of granular deposits in the renal glomeruli which can be shown to comprise DNA and immunoglobulin. In later stages of immunization, only immunoglobulin is found in the glomerular deposits, suggesting that anti-idiotypic antibodies corresponding with the idiotypic determinants on the anti-DNAs may represent another sort of circulating immune complex.

Immune complex disease occurs when the complexes are not rapidly and effectively removed from the circulation. Immune complexes are removed by at least two different mechanisms. The first is ingestion of the complexes by cells of the mononuclear phagocytic system such as macrophages, sinusoidal lining cells of the spleen, and Kupffer cells of the liver.[63] The cells fix immune complexes through their Fc receptors and then endocytose them. The second mechanism for removal of complexes is filtration through the glomerular membrane.[64] This occurs when the complex is small as a result of formation in antigen or antibody excess.

Experimentally, immune complex disease can be produced locally or systemically. The classical example of local immune complex disease, the Arthus reaction, is produced when antibody circulating in the bloodstream encounters antigen injected interdermally.[65] Within a few hours, immune precipitates are formed in the tissue spaces which activate complement and

induce the release of chemo-attractants. Their effect is to attract polymorphonuclear leukocytes to the site of injection and to increase permeability of the vascular endothelium. This accumulation of leukocytes followed by release of their toxic products gives rise to an acute necrotizing vasculitis where large amounts of antigen–antibody complexes can be demonstrated in the vascular walls and perivascular spaces.

Systemic immune complex disease is exemplified by the serum sickness syndrome.[66] In its simplest form, an intravenous injection of a large dose of a potent antigen is followed by slow and then accelerated disappearance of the injected antigen. After a brief period, antibody is produced but cannot be demonstrated free in blood due to the formation of antigen–antibody complexes. The main pathogenic consequences of the circulating immune complexes is seen in highly vascularized structures, such as the renal glomerulus. Immune complexes are deposited in the subepithelial layers of the glomerular loops, where they activate complement-inducing glomerular nephritis and proteinuria.

Immune complex formation is a potential problem following immunization with a hCG–diphtheria or tetanus toxoid vaccine. If a unique amino acid sequence of the β-chain is used for immunization, reactions with endogenous peptide hormones are theoretically possible but must be considered highly unlikely. However, should pregnancy ensue in a vaccinated subject, hCG antigen–antibody complexes may be formed. Immune complex formation involving the protein carrier is also a potential risk as many recipients of the vaccine may have pre-existing antibody to the toxoid from previous immunizations. Moreover, repeated injections will be necessary to maintain the required level of immunity. Subjects will be expected to produce antibodies during the first injection. Subsequent immunizations, therefore, will produce conditions suitable for formation of circulating immune complexes. To date there is no indication that the formation of immune complexes is a significant problem in the use of hCG–toxoid vaccines.

Cytotoxic antibody

Given the frequency of occurrence of autoantibodies, the relative rarity of autoimmune lesions is striking. This observation suggests that the mechanisms for preventing the pathological consequences of autoimmune responses operate successfully in most instances. There is, however, a number of mechanisms by which autoimmune responses may induce tissue injury. Antibody itself is able to produce damage only if the specific antigen is accessible and generally if complement is activated.

Should pregnancy occur in a woman who has received a fertility-control vaccine, the possibility that antibody may affect the fetus must be considered. There is substantial evidence that certain autoantibodies of maternal origin can be transmitted through the placenta in sufficient titre to produce fetal damage. The classical example is haemolytic disease of the newborn, in which

a maternal alloantibody crosses the placenta to produce haemolysis in the fetus or neonate. Other examples are the transient myasthenia gravis found in offspring of myasthenic mothers and the transient hyperthyroidism that occasionally occurs in offspring of mothers with Graves' disease. Currently, a great deal of interest has been aroused by the demonstration that the antinuclear antigen, Ro, antibody produced by the mother can induce heart block in the fetus.[67] Since no such cardiac transmission defect is apparent in the mother, this represents an instance where the autoantibody has an effect on the fetal organ in the absence of any demonstrable action on the corresponding organ of the adult. Thus one must be prepared for the possibility that autoantibodies harmless in the mother may inflict injury on the fetus.

It is also possible that autoantibodies may have indirect detrimental effects during pregnancy. There have been a number of reports, for example, that children of mothers with high titres of thyroid autoantibodies have a greatly increased probability of Down's syndrome.[68] The mechanism behind this observed phenomenon is unclear, but it is possible that autoimmunity in some manner interferes with chromosomal segregation during meiosis. Again, one has to be alert to these possibilities because a fertility-control vaccine may be administered repeatedly.

Autoimmune lesions due to cell-mediated immunity

As explained earlier in this chapter, T cells do not respond to antigen in its native state or in solution. T cell receptors recognize sequential fragments of processed antigen in conjunction with products of the MHC in the surface of APC. Subpopulations of T cells differ somewhat in their functional properties. Some CD4+ cells cooperate with B cells in antibody production or produce the lymphokines responsible for inflammatory and delayed hypersensitivity reactions. These T cells co-recognize antigenic determinants with class II MHC products. In contrast, CD8+ T cells have specific cytotoxic capacity, acting on target cells presenting appropriate antigenic fragments with class I MHC products. They also have non-specific effector activity by the secretion of gamma-interferon. CD8+ T cells are also the major population responsible for antigen-specific suppression.

Vaccines that activate T cells may be associated with a number of possible immunopathological consequences. T cells may respond to the hCG peptide itself or to the protein carrier. The most frequently used vehicle-adjuvant is complete Freund's adjuvant, in which killed acid-fast bacteria are included in a water-in-oil emulsion. The vehicle-adjuvant provides both a depot that slowly releases antigen and a focus for attracting and activating inflammatory cells. The depot prolongs and heightens the immune response. Activated inflammatory cells more readily process and present antigen to T cells and release interleukins that enhance the process of immunization. Complete Freund's adjuvant, therefore, favours a vigorous T cell response.

Individual epitopes resulting from antigenic processing differ in their affinity or dissociability from MHC products.[69,70] The chemical and physical properties determining MHC epitope complex formation are now being elucidated, and certain epitopes are designated 'T cell determinants'. They are often amphipathic, that is, presenting both hydrophilic and hydrophobic regions.[71] The presence of such determinants on an antigenic molecule favours a vigorous T cell response to the molecule.

The engagement of an active T cell response by a vaccine entails both beneficial and harmful potentials. T helper cells promote antibody production and isotype switch, engendering higher levels of IgG antibody which enters tissue spaces and traverses the placenta relatively easily. (Unfortunately, a further switch to IgE class antibody, also favoured by IL-4 production, may increase the risk of immediate hypersensitivity reactivity.[72]) Long-lived T helper cells also promote immunological memory and give rise to an anamnestic response on second encounter with antigen. Other T cells encountering the corresponding antigen release inflammatory mediators of delayed hypersensitivity and granuloma formation which may be responsible for local tissue injury.

Cytotoxic T cells may also be generated during immune responses involving T cell determinants. These lymphocytes damage target cells bearing the corresponding antigen if it is located at the cell surface in conjunction with a syngeneic MHC I product. If gamma-interferon is generated during the cell-mediated response, it may increase expression of MHC class II product on nearby tissue cells, perhaps conferring or enhancing an ability to present autologous cellular antigens.

An hCG subunit vaccine may contain only discontinuous, conformational determinants and therefore not evoke a strong T cell response. None of the immunopathological hazards associated with T cell responses to the self-antigen itself should ensue. The carrier protein expresses T cell determinants, however, and one can expect to produce a vigorous immune response to it with memory.

Assessment of hazards

Tests to be performed in primates prior to the initiation of human trials

An extensive discussion of these tests has been published previously and is summarized in Table 8.3.

Tests on human subjects undergoing clinical trials

Evaluation

An algorithm for the systematic study of human subjects is given in Figure 8.1. The initial critical step in determining the immunological hazards of any

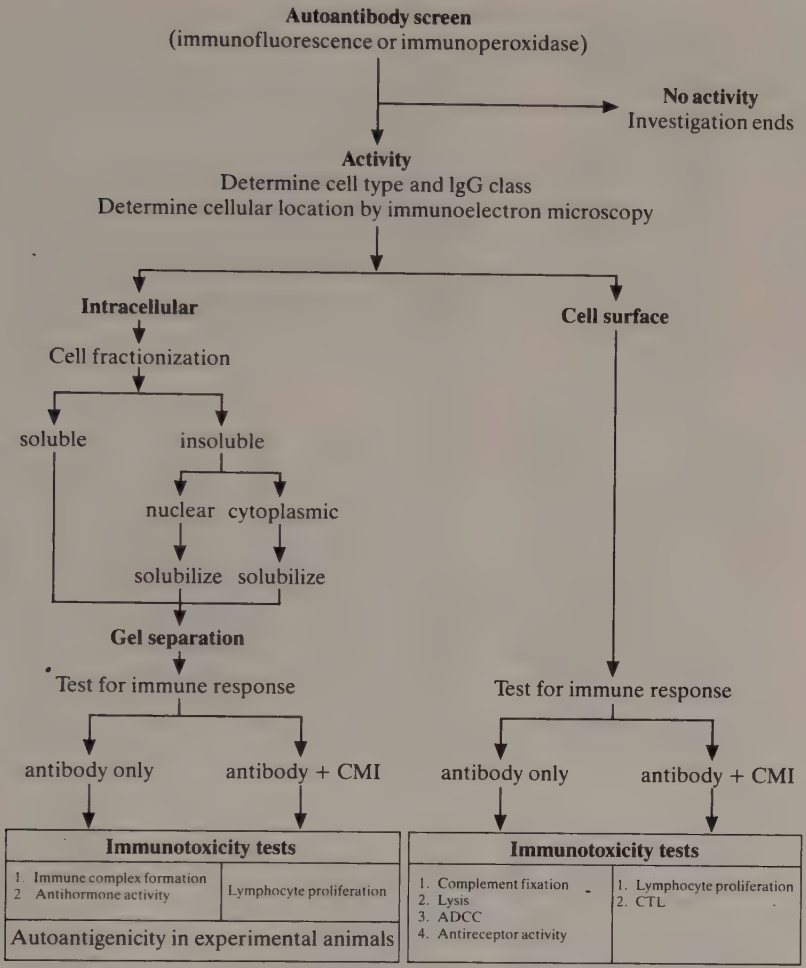

Fig. 8.1 An algorithm for assessing the immunological hazards of an antifertility vaccine. (CMI = cell-mediated immunity; CTL = cytotoxic T lymphocytes; ADCC = antibody-dependent cell-mediated cytotoxicity.)

antifertility vaccine is a survey of the autoantibodies produced. Since the nature of the antigen is initially unknown, immunofluorescence or immunoperoxidase is the method of choice. Not only can a large number of tissues be conveniently tested without the need for extracting, and thereby possibly altering, the antigen, but the reaction defines the location of the antigen. If a positive reaction is found, a number of different tissues should be tested in order to gain information about the distribution of the antigen. The titre of the antibody and its immunoglobulin class help to distinguish induced from 'natural' antibodies. The IgG subclass also predicts whether the antibody fixes complement. This information can be supplemented by immunoelectron

Table 8.3. *Tests to be performed in primates prior to initiation of phase 1 human trials*

Objective	Animal groups to be tested	Nature of test	Timing, duration and frequency of test
1 Monitoring the humoral immune response	Minimally immunized baboons Maximally immunized baboons No-treatment control baboons Adjuvant-only control baboons Adjuvant and carrier control baboons Maximally immunized chimpanzees	(a) Direct antibody-binding of homologous antigens (hCG conjugates, etc.) (b) Neutralization of hCG by antibody	(a) 3, 5 and 7 weeks after immunization, monthly thereafter (b) 3, 5 and 7 weeks after immunization, monthly thereafter
2 Monitoring the cellular immune response	Minimally immunized baboons Maximally immunized baboons No-treatment control baboons Adjuvant-only control baboons	(a) Skin test for DTH (b) Macrophage-migration inhibition (MIF) (c) Lymphocyte blastogenic test (LBT)	(a) Twice within first 6 months after immunization (b) 3 weeks after immunization (c) 3 weeks after immunization
3 Evaluating the risk of cross-reactions and autoimmunity	Minimally immunized baboons Maximally immunized baboons No-treatment control baboons Adjuvant-only control baboons Adjuvant and carrier control baboons Maximally immunized chimpanzees	(a) Direct antibody binding of heterologous antigens (hormones and other purified antigens) (b) Serum levels of hormones (LH, etc.) (c) Antibody neutralization of biological activity of other hormones (d) Immunofluorescence (IF) of sections of normal baboon tissue (e) Complement fixation (CF) with normal baboon tissue extracts	(a) Peak titre and after 6 months post-immunization (b) Peak titre and after 6 months post-immunization (c) Peak titre and after 6 months post-immunization (d) Peak titre and after 6 months post-immunization (e) Peak titre and after 6 months post-immunization

4 Evaluating the risk of immune-complex disease	Minimally immunized baboons Maximally immunized baboons No-treatment control baboons Adjuvant-only control baboons Adjuvant and carrier control baboons	(a) Detection of immune complex in serum: (i) Clq solid phase RIA (ii) Raji cell assay (iii) Radial immunodiffusion for C3, C4 (b) Detect antigen and molecular size of circulating immune complex (c) Detection of immune complex deposits and kidney injury: (i) IF analysis of kidney biopsies (or autopsy) for globulin and complement (ii) Histopathological evaluation of kidney biopsy (or autopsy) (iii) Urine analysis	(a) 2 and 7 days after each immunization and after 6 months (b) Each 6 months after immunization (c) Weekly during first 6 weeks of immunization
5 Evaluating the risk of anaphylactic hypersensitivity	Minimally immunized baboons Maximally immunized baboons No-treatment control baboons Adjuvant-only control baboons Adjuvant and carrier control baboons	(a) Anti-hCG IgF antibody levels (RAST) (b) PK test in baboons (c) Skin-testing for anaphylaxis and Arthus reaction	(a) Peak titre and at 6 months after immunization (b) Peak titre and at 6 months after immunization (c) Peak titre and at 6 months after immunization
6 Evaluating the risk of cross-reaction and autoimmunity	Normal baboons passively administered anti-hCG peptide antibody	(a) Passive administration of ^{125}I-labelled purified anti-hCG antibody; follow-up of elimination	

microscopy, which will give more precise definition. If the antigen is located at the cell surface, it may be the target of autoimmune attack by antibody or by cytotoxic T cells. Intracellular antigens entail mainly the hazards of immune complex formation or antihormone activity or anti-enzyme activity.

If no reaction is found in this initial screen, the vaccine can be considered safe from an immunological point of view, since cell-mediated immunity is rarely found in the absence of antibody under these circumstances. Should antibody be found only to intracellular antigens, a test for antihormone activity is mandatory. If it is negative and resources are limited, a decision may reasonably be made to proceed continuously with clinical testing, since the risks of immunological damage are minimal.

Evidence of antibody to a cell-surface antigen is an indication for further testing. An important question is whether cell-mediated immunity is present. Unfortunately, tests of cell-mediated immunity are relatively difficult, especially if the antigen is poorly defined. Although skin tests are the simplest method of measuring delayed hypersensitivity, they are usually avoided in human trials because of the risk of sensitizing subjects to the antigens under study. Therefore in-vitro tests are performed, such as lymphocyte blastogenesis, or production of lymphokines or interleukins. If cell-mediated immunity is found, attempts to demonstrate cytotoxic T cells can be undertaken with appropriate tissue target cells. If only antibody to the cell surface is detected, tests for possible immunotoxic effects include complement fixation, complement-mediated lysis, and antibody-dependent cell-mediated cytolysis. A positive reaction in any of these tests is a warning sign of a pathogenic immune response.

In the case of a reaction with intracellular antigen, the index of suspicion of a harmful reaction is less. It is, however, useful to define the incriminated antigen. The strategy to be used depends upon whether the antigen is soluble in physiological solution. Soluble antigens are most readily separated in gels on the basis of their molecular size or charge. In addition to related molecular weight, limited information about the chemical nature of the antigens can be obtained. Western immunoblots, combining gel separation with an immunological reaction, are especially convenient and informative. In the case of insoluble antigens, an initial separation into nuclear and cytoplasmic fractions is usually necessary. The nuclear fraction is first separated and ruptured. The soluble portions are analyzed as described above, and the sediment collected, and handled like the other cell particulates. Particulate antigens must be solubilized before proceeding with their characterization. Detergents, organic solvents and chaotrophic ions represent alternative methods for rendering cell particles soluble, thereby permitting gel separation and further characterization.

The presence of cell-mediated immunity to the intracellular antigen increases the likelihood of immunological tissue injury. The in-vitro tests of

lymphocyte proliferation and lymphocyte interleukin production are appropriate in this regard. In the absence of cell-mediated immunity, the major risks are immune complex formation and inhibition of hormone or enzyme functions.

Following identification of the antigenic target of an immunological response, the essential question to consider is whether it has the potential to induce a pathogenic autoimmune response. The question may be answered by using a relevant homologous antigen animal model system and challenging the animals by experimental immunization, usually taking advantage of the potentiating action of Freund's complete adjuvant. Because of genetic difference in susceptibility to autoimmune disease, a variety of inbred strains of rats or mice should be used in these studies, where appropriate.

Genetic risk factors

One goal of a clinical trial of a fertility-regulating vaccine is to identify individuals at greatest risk of adverse consequences. A number of such risk factors might be considered. HLA phenotyping of both Class I and Class II antigens provides some information on the genetic regulation of the immune response. HLA genotypes of subjects in a trial might eventually correlate with the magnitude of the immune response and the development of adverse immunological consequences of vaccination. The pre-existence of a large number of autoantibodies in significant titre may be taken as an indication that the patient possesses an 'autoimmune diathesis', that is, a predisposition to the development of autoimmune disease generally. Tests of a panel of autoantibodies, such as antinuclear antibodies, thyroid antibodies, smooth muscle antibodies, mitochondrial antibodies and striated muscle antibodies, are useful indicators of such a predisposition to autoantibody production. In the case of fertility-regulating vaccines, pre-existing autoantibodies to antigens of the reproductive tract or of peptide hormones may provide better clues. Finally, family histories of certain diseases with definite genetic predisposition, such as autoimmune endocrinopathy, may prove valuable predictors for adverse immunological effects.

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9 Clinical parameters in pre- and post-registration assessment of vaccine safety and efficacy

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Introduction

This chapter will deal with the clinical-phase assessment of antifertility vaccines with particular reference to a method directed against the hormone, human chorionic gonadotrophin (hCG), which recently completed a Phase 1 trial in women.[1] The rationale and principles underpinning the assessment of safety and efficacy of such vaccines have been enunciated in Chapter 1. Here, consideration is only given to an injectable vaccine. If this is successful and a level of immune response correlating with antifertility efficacy can be established, it could be desirable to progress to an orally administered vaccine. There are several vector systems being investigated now which might enable the achievement of this objective.

In terms of both concept and intended mode of action, vaccines directed against the physiological process of conception pose complex and novel problems in vaccine development. Until recently, national drug regulatory authorities have had no specific criteria for dealing with antifertility vaccines, over and above their basic guidelines for the assessment of biological agents and drugs, but they had access to relevant information by consultation with appropriate experts and had extensive experience with antifertility drugs in general. Experience with the anti-hCG vaccine based on the C terminal 37 amino acid peptide of β-hCG coupled to diphtheria toxoid, indicated that an ongoing process of consultation, both with scientists involved in the development of the vaccines and with independent relevant experts, will lead progressively to an appropriate regulatory framework for the initiation, assessment and ongoing surveillance of clinical trials leading to market availability of these novel preparations.

Additional complexities (and expense) in the development of antifertility vaccines include the special requirements for preclinical immunosafety testing in appropriate animal models (usually non-human primates), and the sophisticated and extensive safety assessments required, at least in Phase 1 trials, to detect any potential untoward interactions between the immune, reproductive and endocrine systems and to monitor for cross-reactive autoimmunity.

As evidenced by the passage of anti-hCG vaccine(s) into clinical trials, it is not essential (or required by drug regulatory authorities) that the precise

mode(s) of action of antifertility vaccines be established prior to initial human administration. It is necessary, however, that the measurement of one or more parameters of the immune response to a vaccine in Phase 1 assessment must give at least an indirect indication of potential efficacy. In most instances this will involve the measurement of the levels and biological activity of relevant antibodies, and relating these to efficacy by theoretical calculation and/or by extrapolation from preclinical animal studies.

Quality assurance may pose special problems in clinical trials, particularly if complex vaccine delivery systems are utilized for injectable vaccines. This aspect must be complemented by clinical surveillance for local reactions, and for evidence of variable release rates of components from the injection site. In addition, the possible occurrence of side-effects related to non-antigen components (e.g. carriers, adjuvants, vehicle) must be monitored. Further uncertainties and potential untoward effects may be introduced when antifertility vaccine development embraces the modern revolution in vaccinology, with the use of recombinant antigens, live vectors, and new slow-release delivery systems.

Effective onset, duration of action and potential reversibility must also be assessed comprehensively in a Phase 1 trial of an antifertility vaccine, and taken into account in the design of later phase evaluations. As with any fertility-regulating method, reproductive performance and teratology studies in animal models must precede clinical trials of efficacy. The possibility of immunopathological effects on fetal development must be taken into account alongside the standard requirements for 'pharmaceutical' teratology testing. This poses major problems because the only relevant animal models for such effects are infra-human primates. The use of standard tests in small animals is irrelevant for testing for possible immunoteratological effects of a vaccine. However, non-immunogenic components, e.g. adjuvants, can be tested in small animals. It is suggested that consideration must be given to modifying teratological testing methods for antifertility vaccines.

Clinical trials: general considerations

The general principles applying to the conduct and objectives of clinical trials, and post-registration assessment of antifertility vaccines, are based on those established for steroidal contraceptive drugs, including long-acting injectable preparations,[2] with appropriate modifications dictated by the mechanism(s) of action, route of administration, and duration of effect of a particular vaccine.

Conventionally, the testing of all drugs in the human up to the point of registration (marketing permission or general release) has been divided into three phases.

Phase 1. Studies on the pharmacology of the drug in healthy volunteers.

Phase 2. A: Studies on the pharmacology of the drug in patients.
B: Definitive dose-finding studies.
Phase 3. Extended clinical trials in patients for clinical efficacy, acceptability, and assessment of the extent of side-effects.

The major objectives of Phase 1 and 2 studies are:

(a) to demonstrate a pharmacological action in humans which is likely to be useful in the treatment of specific diseases;
(b) to characterize the dose (concentration)–response curve with special emphasis on interindividual variability and margins of safety;
(c) to devise dosage schedules to be used in the continued clinical evaluation of the drug;
(d) to devise and validate methods for monitoring subjective and objective drug response in these later phases of evaluation;
(e) to monitor adverse effects, both clinical and biochemical.

The objective of Phase 3 studies is to expand the preliminary information generated on the possible mechanism of action and adverse effects, and to ascertain the efficacy and safety of the vaccine in a chosen dose schedule under field conditions. This information provides the basis for introducing the drug into family planning practice in field trials in various settings, as a prerequisite to registration with drug regulatory agencies. Field trials are conducted in a specific programme setting and are designed to confirm the acceptability, use effectiveness, continuation of use, and side-effects (tolerance and management), and to evaluate specific service-related needs and problems.

The recommended sequence of clinical testing of fertility-regulating agents in relation to animal testing is listed below. Further details of animal studies are given in Chapter 3. Comment will also be made on the relevance, or otherwise, of these general recommendations to the testing of an antifertility vaccine as exemplified by past[1,3,4] and planned[4,5] development of an anti-hCG vaccine.

(a) Initial toxicity studies in rodents; pharmacokinetic studies in the most suitable animal species, e.g. a non-human primate. (Including immunosafety studies in a non-human primate exhibiting an antigen with which antibodies to the putative human target antigen will react.)
(b) Phase 1 and 2A clinical testing; studies on drug metabolism. (Including immunogenicity assessment of the vaccine with studies of the nature and kinetics of the immune response to the target antigen.)
(c) Repeated-dose toxicity and reproduction studies in animals. Multiple (hyper-) immunization schedules will have been performed in the preclinical phase to assess immunosafety rather than high- or repeated-dose pharmacological effects. Reproduction studies may require routine rodent teratology as well as immunoteratology

assessment in an appropriate animal model (e.g. baboons for the anti-hCG vaccine).

(d) Phase 2B clinical testing.

(e) Initiation of long-term carcinogenicity studies. (Additional studies may need to be designed to assess any potential carcinogenic effect of the modulation of the immune system by vaccination – general experience with vaccines would suggest that this is a highly unlikely possibility.)

(f) Phase 3 clinical testing.

(g) Specific studies on biochemical variables and physiological (and immunological) events.

Clinical trials of antifertility vaccines

There is limited information available on the conduct or results of formal clinical trials of antifertility vaccines. Four different vaccines based on the principle of immunization against hCG have now reached the stage of clinical testing.[1,6–9] What follows in this section derives largely from experience accumulated in initiating a Phase 1 trial,[1,10] and preparing for a Phase 2 trial, of one of these vaccines. Specific requirements for antifertility vaccine trials will be formulated within the framework provided for steroidal contraceptives.[2] These requirements will differ to a certain extent depending on the nature of the vaccine, its target antigen, mode of action, composition, route of administration, number of doses required for primary immunization, duration of effect, timing of booster immunizations, and predicted possible adverse effects based on pre-clinical studies. These differences will be relevant particularly to Phase 1 and Phase 2 studies.

Steps to a Phase 1 clinical trial

The novelty of vaccination as a means of fertility regulation has implications for several aspects of the preparatory stages of a Phase 1 trial. Some of these aspects (legal, ethical and regulatory) will be dealt with in more detail and broader prospective in Chapters 12 and 13, but deserve comment here.

Ethical aspects

The ethical principles guiding the conduct of biomedical research involving human subjects are laid down in the Declaration of Helsinki of the World Medical Association as revised by the 29th World Medical Assembly in Tokyo in 1975 and in the 'Proposed international guidelines for biomedical research involving human subjects'.[11] All studies should be performed on volunteers, who, in Phase 1 trials, may receive no therapeutic benefit from the agent administered. The volunteers must give informed consent, after being advised of the risks and benefits of the study. For an antifertility vaccine, in Phase 1 and 2 studies, they must understand that reversibility cannot be assured.

Unlike strictly therapeutic drugs, a contraceptive method is intended for use by healthy individuals for prolonged periods of their reproductive life, rather than for the treatment of specific disease. This imparts an added dimension of ethical responsibility to the conduct of clinical trials of contraceptives: an imperative that assumes an even sharper focus when vaccination against reproduction is contrasted with vaccination against infectious diseases, which may be life-threatening on a global scale. Moreover, more frequent, repeated immunization may be necessary to maintain contraceptive levels of immunity.

The 'international guidelines' state that a clinical trial and its protocol should be reviewed by an independent ethics committee. In practice, it is likely that an antifertility vaccine will require ethical approval at several levels prior to a Phase 1 trial: for example, an international agency, if such is involved as sole or joint sponsor of the trial; a national body; and an institutional ethics committee.

Legal indemnity

In view of the novelty of antifertility vaccines, investigators will be wise to clarify the nature and extent of their legal indemnity and of subject compensation in relation to, particularly, early phase trials. They should also seek confirmation, in writing, of the extent of legal responsibility for trial mishaps which will be accepted by each of the involved parties, which may include international agencies, pharmaceutical companies, government agencies and other institutions.

Drug regulatory agencies

The regulatory aspects of the development, clinical assessment and marketing of antifertility vaccines are considered in Chapter 12. Several national agencies have already been involved in approving Phase 1 trials of anti-hCG vaccines. Some of the difficulties they have experienced have been alluded to in the introduction to this chapter. The 'learning curve' for the processing of clinical trial applications for such novel products will no doubt continue for some years. However, precedents have already been set, and the sharing of information on protocol format between investigators, and on assessment guidelines and strategies between national regulatory agencies which has begun, should be encouraged. In this way the progression of future vaccines through the regulatory process may be facilitated.

Recruitment of subjects

The recruitment of subjects for a Phase 1 (immunogenicity and safety) clinical trial of an antifertility vaccine is logistically difficult and time consuming. Detailed explanation is required for volunteers and staff involved, covering the nature of the vaccine, the aims of the trial, the criteria for inclusion in the trial and the commitment necessary for successful completion. Potential volunteers may be identified by approaches to selected community groups, for example family planning clinics and women's organizations. It is also helpful

to encourage sympathetic media coverage. As a result of this strategy, recruitment for the anti-hCG vaccine trial in Australia[1] resulted in 181 telephone enquiries from female volunteers, of which 89 were potentially suitable for inclusion. Of these, 57 were interested in participating in the trial, and the final group selected for screening numbered 43 to provide for 30 subjects plus reserves.

Subject selection

Certain of the criteria for subject selection vary with the nature of the vaccine, and whether it is directed at males or females. In general terms, the age range of subjects is more restricted, and the selection criteria are more conservative for a Phase 1 trial than for subsequent studies. The phase-related requirements of a trial also predetermine the baseline subject selection criteria.

Broadly, a Phase 1 trial requires volunteers who are not at risk of pregnancy, so that safety, immunogenicity, dose-responsiveness and potential efficacy can be assessed. Optimally, they should be of reproductive age, no younger than 25 years, and have had children previously. These criteria take some account of the unproven potential reversibility of vaccines entering Phase 1 studies. Unfortunately, these optimal criteria are difficult to attain in most study populations. Phase 1 subjects will usually be required to have been surgically sterilized or be reliably contracepting using a method, such as the intrauterine device, which does not interfere with either the activity of the vaccine or with the assessment of laboratory and clinical parameters. Hormonal methods are unacceptable because of the possibility that they might influence immune responses and, in the female, because they will mask menstrual irregularities, fail to allow monitoring of ovulation, and disturb hormonal and biochemical indices.

There might also be circumstances where Phase 1 studies could appropriately be conducted in other categories of subjects, such as post-menopausal women, or in the opposite gender to that in which the vaccine is designed to operate. Such studies, however, whilst providing immunogenicity data, would only yield limited information on safety aspects.

Beyond Phase 1, subjects would be required to be potentially fertile healthy individuals in whom efficacy and acceptability can adequately be assessed and who are prepared, at least in Phase 2 studies, to co-operate in the assessment (either direct or indirect) of reversibility. Couples participating in Phase 2 and 3 trials should be offered the option of pregnancy termination if an unwanted pregnancy occurs, provided such a procedure is legally permitted and ethically acceptable in the trial location.

Subject selection for a Phase 1 trial

The following criteria were employed in a Phase 1 anti-hCG vaccine trial[1] and serve as a guide for other vaccine trials.

(i) *Inclusion criteria.*
Healthy female volunteers with a normal weight/height ratio. Premenopausal and over the age of 29 years.
Regular menstrual cycles of length 25–36 days for at least three consecutive cycles.
Willingness to abide by the protocol and to present for physical examinations, injections, blood sampling and other tests at prescribed times.

(ii) *Exclusion criteria.*
Pregnant or breast feeding.
History of endocrine disease.
Receiving hormone or prolonged antibiotic therapy or other regular medication.
Personal or family history of severe, acute or chronic allergies.
History of idiopathic urticaria or angioedema.
History of reactions to previous immunizations.
History of significant anti-drug reactions.
History of reactions to x-ray contrast media.
History of unexplained recurrent skin rashes.
History of dry eyes and mouth.
History of complicated childhood exanthemata.
History of complicated rheumatic fever.
Raynaud's syndrome.
Arthritis.
Malignant disease.
Renal, hepatic or cardiovascular disease.
Autoimmune disease.
Abnormal laboratory tests.
Tuberculosis, malaria or parasitic infestations.
Abnormal ECG.
Addiction to drugs.
Family history (one or both parents or siblings) of insulin-dependent diabetes.
Family history (one or both parents or siblings) of autoimmune disease.
Positive skin (prick) test to diphtheria toxoid.
HLA B27 positive.

To these exclusion criteria should now be added: positive human immunodeficiency virus (HIV) antibody or hepatitis B surface antigen tests or refusal to be tested.

This list requires obvious modification for male subjects, and with regard to the specific requirements of other vaccines. For example, the diphtheria toxoid skin test is specific for the anti-hCG vaccine in order to exclude acute hypersensitivity to the carrier. HLA B27 subjects were excluded from this trial

because of the (largely theoretical) risk that they might be susceptible to adjuvant-induced arthritis. This precaution was probably unnecessary and, of course, is irrelevant to vaccines which lack an adjuvant.

Subject selection for a Phase 2 trial

Similarly, criteria have also been established for a Phase 2 trial of the same vaccine.[12]

(i) *Inclusion criteria.*
Healthy female volunteers.
Aged between 18 and 35 and pre-menopausal.
Regular menstrual cycles of between 25 and 36 days.
Having had three consecutive cycles of normal length immediately prior to treatment, of which two must have been ovulatory.
Having had one proven pregnancy.
Willing to rely solely on this treatment as a method of fertility regulation during the exposure period of the study.
Engaged in a stable relationship with a non-contracepting, non-vasectomized male and exposed regularly to the risk of pregnancy.
Willing and able to keep a menstrual diary and adverse reaction record.
Willing to abide by the protocol.
Willing and able to present at the prescribed time for treatment and follow-up.

(ii) *Exclusion criteria.*
Pregnant or lactating.
Two or three pre-treatment cycles anovulatory as determined by mid-luteal measurements.
Personal or family history of severe, acute or chronic allergies, idiopathic urticaria, angioedema, significant asthma or eczema.
History of severe reactions to previous immunizations; significant anti-drug reactions; reactions to x-ray contrast media.
History of complicated childhood exanthemata.
History of sexually transmitted disease.
History of recurrent abortions.
Previous child with significant congenital anomalies.
Rheumatoid arthritis, malignant disease, autoimmune disease, renal, hepatic, or endocrine disease, including diabetes.
Active tuberculosis, malaria or parasitic infestations.
Addiction to drugs.
Abnormal ECG.
HIV antibody positive or refusal to be tested.
Hepatitis B surface antigen positive.
Positive skin test to diphtheria toxoid.

Subject selection for Phase 3 and field trials

Inclusion criteria for large-scale multicentre trials of antifertility vaccines are no different from those applying to similar trials of any contraceptive agent and are based on the same principles as those of Phase 1 and 2 trials. Exclusion criteria beyond Phase 2 trials are markedly truncated, and include only those conditions which contraindicate contraception in general (e.g. pregnancy), and vaccination in general (e.g. immunosuppressive or malignant disease), together with disorders posing a specific method-related risk. These must be minimal otherwise the utility of the method will be severely diminished. There are no method-related contraindications for an anti-hCG vaccine. In moving from a Phase 3 study to larger field evaluations, as subject-selection criteria are minimized, monitoring for adverse clinical effects is essential. Random small samples of the study population should be evaluated both clinically and biochemically, as well as carrying out a general epidemiological surveillance.

The construction of clinical trials of antifertility vaccines

It is beyond the scope of this chapter to enumerate the fine details of clinical trial protocols for each Phase of testing of the range of current and future antifertility vaccines. The nature and requirements of Phase 1, 2 and 3 trials will be described, together with an outline of protocol construction with specific reference to an anti-hCG vaccine. Reasonable detail will be included, however, to indicate the scope of monitoring required to detect relevant side-effects and to assess efficacy.

Phase 1 trials

A Phase 1 trial of a contraceptive vaccine is designed to assess immunogenicity, dose levels and safety in a small group (up to 50) of subjects. The subject involvement may be intensive and require strong motivation and commitment. Comprehensive hospital-based clinical and laboratory facilities are required. The intensity of monitoring may need to be modified to accommodate unavoidable geographic, cultural and logistic difficulties,[8] and to avoid an unacceptable imposition on the health status of volunteers. For example, while a total of 400 ml of blood was sampled over a 9-month period in Phase 1 trial subjects in Australia, such an extensive sampling scheme might be inappropriate in other populations. Given that these caveats must apply to individual local situations, the Phase 1 trial description which follows should not be viewed as an optimal protocol design but one which may be simplified with further experience. Once again the protocol described derives from an anti-hCG vaccine trial conducted in Australia.[1]

Following screening and counselling, subjects were given written information about the trial and signed a consent form. They then entered the trial

Table 9.1. *Protocol outline for a Phase 1 trial of an anti-HCG vaccine*

Day 0	Screening, menstrual calendar for 3 cycles
Day 1	First injection, left gluteal muscle; 48 hours hospitalization; clinical monitoring; laboratory tests; inspection of injection site
Days 7 & 14	Clinical assessment; laboratory tests
Week 5	Clinical assessment; laboratory tests; DT skin test
Week 6	Second injection, right gluteal muscle; 48 hours hospitalization; clinical monitoring; laboratory tests; inspection of injection site
Weeks 7, 8, 12	Clinical assessment
Month 4	Laboratory tests
Month 6	Full physical assessment; laboratory and clinical tests
	Further follow-up as indicated

and commenced a menstrual diary. The basic design of this trial comprised a 3-month pretrial period, a 6-month study phase, and further follow-up as indicated. The vaccine consisted of a synthetic 109–145-amino-acid peptide of the C-terminal region of β-hCG, conjugated to diphtheria toxoid, and dissolved, along with a synthetic muramyl dipeptide as adjuvant, in the aqueous phase of a squalene-mannide mono-oleate-saline emulsion. The subjects were distributed in five dosage groups of six each, and received the vaccine by deep intramuscular injection on two occasions, 6 weeks apart. In each dosage group, four subjects received the full vaccine, and two received the vehicle and adjuvant alone, in a similar injection volume. In order to minimize the risks in each treatment group, injections were given in a sequential manner to allow detection of, at least, early side-effects, before moving to a higher dosage group. An outline of the trial protocol is shown in Table 9.1.

At entry into the trial, a full history was taken and a physical (including pelvic) examination was performed together with the following investigations.

Cervical smear
ECG
Bleeding time
Ophthalmoscopy
Urinalysis
Microurine examination
Routine haematology:
  haemoglobin
  haematocrit
  RBC
  total and differential WBC
  platelet count
  erythrocyte sedimentation rate (ESR)
Special haematology:
  prothrombin time

- activated partial thromboplastin time
- fibrin degradation products

Direct Coombs' test

Biochemistry:

- electrolytes
- urea
- creatinine
- bilirubin
- alanine aminotransferase (ALT)
- serum glutamic oxaloacetic transaminase (SGOT)
- alkaline phosphatase
- gamma glutamyl transferase (GGT)
- total protein
- albumen
- cholesterol
- triglycerides
- glucose
- calcium
- inorganic phosphorus
- creatine kinase (CK)

Hormones:

- thyroxine (free T4)
- cortisol
- oestradiol
- follicle-stimulating hormone (FSH), luteinizing hormone (LH)

Immunology:

- complement levels (C'3, C'4)
- immunoglobulins (G, A, M)
- immune complexes
- antinuclear antibody
- rheumatoid factor
- tissue autoantibodies against:
  - thyroid, gastric parietal cells, mitochondria, smooth muscle, striated muscle, pancreas, pituitary
- immune response tests:
  - anti-hCG
  - anti-β-hCG-C-terminal peptide
  - anti-FSH, LH, prolactin, thyroid-stimulating hormone (TSH)
  - anti-diphtheria toxoid

These procedures and investigations were repeated at intervals during the trial, as indicated in Table 9.2. In addition, at each of these assessments, the subjects were questioned about side-effects, menstrual disturbances, and evidence of hypersensitivity reactions (e.g. joint and skin symptoms, lymphadenopathy).

Table 9.2. *Protocol for investigations in a Phase 1 trial of an anti-HCG vaccine*

Procedure	Day 0	Day 1[a]	Day 7	Day 14	Week 5	Week 6[a]	Week 7	Week 8	Week 12	Month 4	Month 6
Full physical examination & vaginal cytology	X										X
Interim physical examination		X	X	X	X	X	X	X		X	
ECG	X			X				X			X
Ophthalmoscopy	X				X			X		X	
Bleeding time	X		X				X			X	
DT skin test					X						
Urinalysis	X	X	X	X	X	X	X	X		X	X
Microurine	X	X	X	X	X	X	X	X		X	X
Routine haematology	X	X	X	X	X	X	X	X		X	X
Special haematology	X	X		X		X		X			
Biochemistry	X fast	X fast	X fast	X	X	X fast	X fast	X		X	X
Endocrine assays	X		X							X	
Immunology	X				X		X				X
Direct Coombs' test	X				X		X				X
Immune response tests	X	X	X	X	X	X	X	X	X	X	X

Note:
[a] Injections were given at Day 1 and Week 6.

Acute monitoring was performed over the 48-hour period following each injection. This included:

vital signs,
inspection of injection site,
examination for rashes, oedema and bronchospasm,
urinalysis (at 24 and 48 hours),
microurine examination (at 24 and 48 hours).

Rationale of subject monitoring for safety

The rationale for the comprehensive monitoring schedule incorporated into this trial was to assess the occurrence of adverse effects in three categories.

(i) Non-specific: i.e. unexpected abnormalities in biochemical or haematological parameters.

(ii) Immunological: i.e. immune complex formation (urinalysis, microurine, immune complexes, C′ levels); cross-reactive autoimmunity (autoantibodies, rheumatoid factor, hormone assays, antibodies to pituitary hormones); uveitis (ophthalmoscopy); muscle damage at injection site (CK); hypersensitivity (acute post-injection monitoring).

(iii) Endocrinological: i.e. LH cross-reactivity (menstrual pattern, reproductive hormone levels); TSH and ACTH cross-reactivity (thyroxine, cortisol levels).

Monitoring of efficacy

The potential efficacy and duration of action of the anti-hCG vaccine were assessed by the serial measurement of hCG-binding (anti-hCG antibodies), and hCG-neutralizing capacity, in serum. Theoretical calculations gave an indication of the serum concentrations of antibody that would be necessary to neutralize physiological amounts of hCG likely to be present in blood in the latter part of a conceptual cycle.

Additional information relevant to the utility of the vaccine was gained by anti-hCG isotype analysis, and by in-vitro assays of T lymphocyte recognition and response. The latter indicated the absence of a cellular immune response (and therefore of memory) to the vaccine antigen, and confirmed the likely reversibility of the antifertility effect.

Immune reactions to the carrier (diphtheria toxoid) were also studied and showed clear evidence of both cell- and antibody-mediated responses. Such data may assume importance if repeated booster doses of a carrier-containing vaccine are administered, and are also relevant to the potential prophylactic effects of immunity developing to other carriers such as tetanus toxoid. Further discussion on the importance and relevance of various assays of the immune response to antifertility vaccines is presented in Chapter 6.

Subsequent to the completion of this trial, it has been recommended that HLA typing of subjects may allow an assessment of genetic variations in response to vaccination. This test should now be incorporated into Phase 1 and 2, and possibly Phase 3 trials, of antifertility vaccines.

Phase 2 trials

A Phase 2 trial of an antifertility vaccine should involve using the optimal dose and administration schedule, or acceptable modifications thereof, as determined from the Phase 1 study. The trial is designed to assess efficacy and side-effects usually in around 100 subjects, but these may be multiplied by the use of more than one trial centre. It should be preceded by reproductive performance and teratogenicity studies in animals, and pregnancy termination may be offered, if legally and ethically aceptable, as an option should pregnancies occur. The protocol should provide indices for termination of the study should an unacceptably high rate of pregnancy or adverse reactions occur. Depending on the event leading to such termination, it may be necessary to abandon further development of the product or to reformulate it and/or to re-evaluate it at a different dose level, or in a different administration schedule.

A protocol has been developed[12] for a Phase 2 trial of the anti-hCG vaccine described above. This study will be carried out in two centres with 125 female

subjects participating in each location. Following administration, the women will maintain use of an intrauterine contraceptive device (IUCD) until anti-hCG levels attain a contraceptive level. At this point, the IUCD will be removed, antibody levels will continue to be monitored, and the device will be replaced when they fall close to the protective level. It has been estimated that the immunization schedule will elicit effective levels of antibodies within a reasonable safety margin for at least 3 months in all trial subjects, and perhaps as long as 6 months or more in some individuals. In order that the absence of pregnancies among the study subjects be compatible with a true annual pregnancy rate of at most 5% (upper one-sided 95% confidence limit), a total of approximately 750 cycles of exposure need to be observed. Depending on the duration of theoretically effective immunity, this corresponds to 250, 188, 150 or 125 subjects studied for exposure periods of 3, 4, 5 or 6 months respectively.

The overall duration of the study will be 12 months: 3 months pre-treatment, 3 months to cover the immunization schedule, and 6 months post-immunization efficacy evaluation.

The study will be terminated in any subject who:

(i) wishes to withdraw for any reason;
(ii) fails to attain a satisfactory level of antibody;
(iii) develops a significant study-associated condition;
(iv) becomes pregnant.

The study itself will be terminated if two or more subjects become pregnant with an estimated contraceptive antibody level at the peri-implantation stage of embryonic development.

Any pregnancies occurring during the course of the study and during the subsequent 3 years of post-injection surveillance will be carefully monitored. Offspring will be assessed post-natally at 12 months of age. Particular attention will be paid to those pregnancies that occur in the presence of maternal antibody levels at the 'contraceptive' limit or above, which are not terminated at the request of the mother.

On entry into the trial and at its formal conclusion, a full history and physical examination will be performed together with the following investigations.

ECG
Microurine examination
Urinalysis
Cervical cytology
Routine haematology:
  haemoglobin
  haematocrit
  ESR
  RBC

WBC total and differential
platelet count
Routine biochemistry:
electrolytes
urea
creatinine
alanine aminotransferase (ALT)
alkaline phosphatase
bilirubin
total protein
albumen
Immunology:
immunoglobulins (G, A, M)
complement levels (C'3, C'4)
anti-diphtheria toxoid antibodies
rheumatoid factor
antinuclear antibodies
tissue autoantibodies against: thyroid, gastric parietal cells, mitochondria, smooth muscle, striated muscle, pancreas, pituitary
Hepatitis B surface antigen
HIV antibody
Diphtheria toxoid (DT) skin test.

Certain of these investigations (DT antibody, urinalysis, microurine, DT skin test), together with an interim physical examination, will be repeated on occasions during the trial. A menstrual diary and adverse reaction card will be maintained throughout the trial.

A complex temporal protocol will ensure that the immunization schedule will provide a maximum number of potentially protected cycles, and that the discontinuation and resumption of pre-existing contraception (IUCD) will be correctly timed.

Factors relating to efficacy will be assessed by:

(i) serial luteal phase serum progesterone assays to confirm ovulation;
(ii) serial serum assays for binding and neutralizing antibodies to hCG;
(iii) serological and clinical diagnosis of pregnancies.

The major problems envisaged with this trial are:

(i) the frequent monitoring and blood sampling required;
(ii) the need for a rapid turn-around of results for the anti-hCG assays;
(iii) the logistics of determining and utilizing the 'protective' antibody level;
(iv) concerns about subtle immunoteratological effects if pregnancies are established and continued in the face of detectable maternal anti-hCG antibody levels.

Phase 3 trials

Phase 3 trials of antifertility vaccines will comprise several thousand subjects studied in different centres. Ideally, these trials should be randomized, controlled comparisons between the test agent and an established method of contraception. The studies should be designed with sufficient statistical power to demonstrate a clinically important difference between treatment groups, with regard both to efficacy and side-effects. It is difficult to choose a control method to be used in comparative studies for an antifertility vaccine, but a logical choice might be an injectable steroid or an IUCD.

In any event, if the objective of the study is to differentiate pregnancy rates of 4% and 2%, at 1 year between two groups with a two-sided significance level at $p = 0.05$ and a power of 50%, 560 subjects per group are required, while for a power of 80%, 1140 subjects per group are required. However, if it is necessary to differentiate between pregnancy rates of 0.5% and 0.25%, 4600 and 9400 subjects are required for the powers of 50% and 80% respectively. In practice, it has been demonstrated that the study of up to 1000 subjects, with a minimum of 10 000 months of exposure to a new contraceptive, in comparison with an established method, in a randomized trial, is sufficient to allow an adequate assessment of safety and efficacy prior to registration and wider usage.

Population differences may affect the results of large clinical trials with regard to efficacy and side-effects, and these must be allowed for in the design and subsequent analysis of the study. These considerations are particularly important for vaccines, where the immune response may be modified by genetic variations, and by the influence of intercurrent endemic illnesses. It is important therefore that Phase 3 trials are conducted in several representative populations.

Several other factors of specific relevance to antifertility vaccines require attention in Phase 3 trials, and in subsequent field trials, in which acceptability and utility are put to the test.

(i) The feasibility and acceptability of an antifertility vaccine if more than one administration is required for primary immunization.

(ii) The necessity, or otherwise, to test for protection following primary immunization and as a guide to the need for and timing of booster immunizations. A requirement to obtain a venous blood sample for these purposes would severely limit the acceptability of the method. There is an urgent need to develop a simple and relatively non-invasive technique to assess immunity to the vaccine, preferably by self-monitoring. This could involve the use of a simple 'kit-type' colorimetric method of antibody measurement on a finger-prick blood sample or on saliva or vaginal secretions (see Chapter 6).

(iii) In the absence of such a simple test, the alternative need to limit the time interval for boosting to the minimum period required for

efficacy in the Phase 3 clinical trial. This in turn would bear on the logistics of follow-up and return for booster immunizations, and the medium term effects of repeated secondary immunizations – say over a period of 3 years. (Longer term studies would be the province of post-registration surveillance.)

(iv) The need to monitor specific variables or phenomena related to the vaccine response. For example, if serological evidence of potential, but presumably inconsequential, cross-reactive autoimmunity arose in Phase 1 or 2 studies, this might require further examination by clinical review or functional tests in large numbers of users.

Post-registration assessment

It would require a crystal ball of considerable clarity to foreshadow the specific requirements of post-registration assessment of antifertility vaccines. In general, however, the need for continuing post-marketing surveillance of a contraceptive agent has been amply confirmed by experience with oral steroids and intrauterine devices. Post-registration surveillance is necessary because studies in laboratory animals may not predict human toxicity, and Phase 3 trials can detect only relatively common side-effects that occur over a short period during or after use of the method. Five strategies are used for the post-registration surveillance of drugs and therapeutic agents. These are:

(i) the collection and use of reports of adverse reactions;
(ii) large-scale experimental studies;
(iii) formal epidemiological studies (cross-sectional, case-control, and cohort);
(iv) indirect correlation studies; and
(v) investigations of outbreaks.

These methods are complementary, each having its strengths and limitations. All have potential uses in a comprehensive post-registration surveillance scheme.

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10 Issues relevant to other current or proposed vaccination programmes

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Introduction

The development of fertility-regulating vaccines has yielded information of relevance in a number of areas of vaccine development and vaccination programme implementation. In particular, the experience gained in the development and initial clinical evaluation of a vaccine based on a synthetic 37-amino-acid peptide of the C-terminal of the β-subunit of human chorionic gonadotrophin (hCG), coupled to diphtheria toxoid and administered in a novel adjuvant formulation, should prove valuable in other ongoing vaccine development programmes as well as in areas where novel vaccine development is under consideration.

Vaccination against self antigens

Vaccination to control autoimmune disease

As mentioned in Chapter 1, ideas about the nature of autoimmunity and the initiation of autoimmune disease have changed markedly in recent years. Anti-self antibodies are found in a significant proportion of apparently healthy people, though this does not mean that autoantibody does not cause considerable pathology in some situations. However, the ability to induce autoimmune disease in several model systems by the transfer of cloned T cells specific for defined self antigens[1] is switching the emphasis from antibodies to activation of T cells as the initial event in the host response which leads to disease.[2]

Thus, transfer of specific cloned T cells to rats can induce experimental autoimmune encephalomyelitis (EAE) or adjuvant arthritis.[1,3] The mechanism of T cell activation now generally accepted is the occurrence on the target cell surface of peptides associated with major histocompatibility complex (MHC) antigens, the complex being recognized by the T cell receptor of the appropriate specificity. The peptide is a degradation product of the autoantigen which is recognized by the antibody.[2]

Two proposals, deriving from this concept, have been made. The first is based on the idiotype-anti-idiotype network theory. Receptors with enhanced specificity for the peptide–MHC complex on the activated T cell (which have

been clonally selected) should be regarded by the host as foreign. If so, their use as immunogens should generate an immune response, specific to the receptor, which might result in the destruction of the effector T cell. This approach, when applied to rats with EAE, is reported to have caused remission of the disease.[1] There is other evidence that this approach mimics the natural course of the disease in this host. T cells of the CD8 phenotype have been isolated from the spleens of rats that have recovered from T-cell-mediated EAE and which lyse the T cells which induced the disease.[4] Pretreatment of rats with the CD8 cells prevents T cell induction of the disease.

The second proposal is an extension of the observations that two autoimmune diseases, allergic encephalomyelitis[5] and myasthenia gravis,[6] experimentally produced in mice, can be prevented by concurrent injection of anti-Ia sera of the appropriate (i.e. that of the mouse) specificity. Similar treatment can delay the appearance of other diseases, such as murine lupus. However, such treatments are unsuitable as a therapeutic measure as they cause a general depletion of Ia-bearing cells.[7] The proposal[2] is to generate a monoclonal antibody(ies) which was so specific for the peptide–MHC antigen complex characteristic of the disease that other peptide–MHC antigen complexes at the surface of other cells are unaffected. It would be expected, by analogy with the above findings, that treatment with this reagent should result in prevention and/or remission of the disease. If so, an anti-idiotypic preparation might again be appropriate to use as the basis of a vaccine to prevent the occurrence of autoimmune disease.

Blocking the cellular receptors for viruses

A major difficulty in the development of vaccines to control some viral infections is antigenic variation due to changes in amino acid sequence in the 'protective' surface antigens of the virus. Two examples are the haemagglutinin of the influenza virus and the gp120 (env) protein of human immunodeficiency virus (HIV). As the receptor on the virus-susceptible cell would not be expected to show marked sequence variation, one potential approach to overcome this difficulty is to block the site of attachment of the virus with antibody directed against the cell receptor.

Rhinovirus, which is the major aetiologic agent causing the common cold, undergoes antigenic drift (more than 100 'serotypes' are known). Cellular receptors of two antigenic specificities have been identified; about 90% of the viral serotypes binds to one of these two receptors. Two monoclonal antibody preparations showed high affinity for this receptor and blocked infection of cells *in vitro*, even in the presence of 3000 particles/cell.[8] Prolonged culture of the cells in the presence of the antibody did not have any detectable adverse affect. A small clinical trial showed that frequent intranasal administration of the antibody lessened the severity of a challenge rhinovirus infection.[9]

An approach related to this is being used in attempts to prevent HIV

infection. The CD4 molecule on the T cell is the receptor for HIV.[10] Many preparations of anti-CD4 antibodies block infection *in vitro* by different HIV isolates, suggesting that the viral anti-receptor site on the gp120 (env) protein is conserved.[11] Injection of monoclonal antibodies directed to CD4 molecules results in the formation of anti-idiotype antibody which 'mimics' CD4 molecules and presumably competes with the cellular receptor for binding to the viral anti-receptor site.[11]

The alternative approach in both these cases is to immunize the host, using the receptor molecule as the immunogen. If the intact molecule was used, there would be the potential risk of autoimmune disease as both T and B cell responses are likely to be generated. However, if an exposed peptide which did not have the properties of a T cell epitope was chosen and linked to a carrier protein, only antibody production and not T cell activation by the peptide should occur and this might prove to be less hazardous.

Vaccination against tumour antigens

There is a very extensive literature on attempted immunization with tumour-specific antigens (TSA) to prevent or cause rejection of tumours. In many cases, prevention and rejection may depend upon the induction of a cell-mediated immune (CMI) response rather than an antibody response. Some TSAs are secreted so that antibody may be diverted from the important target.

Recently, more encouraging results have been obtained and one will be discussed as an example. Some viral infections directly cause tumorigenesis, and viral antigens are expressed in the malignant cell. A recombinant vaccinia virus preparation containing genes coding for polyoma viral antigens was shown not only to prevent tumour formation by this virus but also to induce rejection of many tumours already established.[12] It is to be expected that results like this will encourage a similar approach towards the common human tumours where the TSA is unlikely to be virus-coded. Monoclonal antibodies to such TSAs are now being used for diagnosis and for imaging of human tumours. Many such TSAs are oncofetal antigens and not entirely specific for the malignant cell, although they may be expressed to a higher level on these compared to other cells. Generation of a CMI response in such cases may run the risk of inducing autoimmune disease, although it is possible effector T cells may not recognize cells expressing very low levels of the antigen.[13,14]

Aspects of more general relevance to other vaccine development programmes

Most vaccines in current use were developed on an ad hoc basis; they were acceptable provided they caused only minor side reactions and no, or very little, mortality. The eradication of smallpox was achieved with a vaccine which was highly effective but which caused significant morbidity and

mortality;[15] this was acceptable at the time because of the severity of the disease and the possibility of eradication. It was unnecessary to know anything about the immune response generated – not even a single immune parameter such as seroconversion – because many decades of use had demonstrated the vaccine's efficacy and because a scar which developed at the injection site was evidence of a successful 'take'. Vaccination with attenuated preparations is still a promising option for the control of some other viral diseases.[16]

Many of the remaining infectious diseases of public health importance offer a much greater challenge. To develop effective vaccines against many parasites and other agents which cause chronic infections such as HIV, it will be necessary to know in detail not only the molecular properties of the agent but also the pattern of replication, changes in the tropism of the agent, the types of immune response generated, which of these is protective, and the extent of immunopathology which ensues. Certainly during the development of many vaccines and possibly also during post-registration surveillance, assays for monitoring these responses will be required and some may need to be quite sophisticated.

A need for specific T cell responses

Some examples can be given of recent findings in a variety of experimental systems which demonstrate the importance of effector T cell responses in providing immunity to a variety of infectious agents. Perhaps the more dramatic findings have come from the study of Plasmodia infections. For many years, the accepted view was that specific antibody was the critical factor in preventing and controlling these infections. Good[17] has reviewed recent findings. An early series of papers by Clark and colleagues,[18,19] which led to the demonstration that reactive oxygen intermediates caused haemolysis and parasite death, may now provide clues to the interpretation of some recent findings.

1. Depletion of CD8+ but not of CD4+ cells in mice previously immunized with x-irradiated sporozoites rendered the mice susceptible to challenge with sporozoites.[20,21] The active agent seems to be gamma-interferon, which, in the case of uncultured cells, is mainly a product of CD8+ cells.
2. A CD4+ T cell clone, which also secretes gamma-interferon, has recently been shown to transfer protection adoptively for a murine malaria parasite.[22] As this acts at the blood stage, it has been proposed[17] that the gamma-interferon may activate monocytes to destroy intraerythrocytic parasites.

It has also now been shown[23] that a monoclonal antibody specific for gamma-interferon eliminated resistance to acute *Toxoplasma gondii* in mice. The activation of macrophages, which normally occurs and which kill

Toxoplasma *in vivo*, was inhibited by administration of the antibody. The presence of parasite-specific antibodies in the infected mice seemed to confer little if any protection.

Several other examples, such as murine cutaneous leishmaniasis, Listeria and BCG infections, in which a class I MHC-restricted T cell response has been shown to be necessary to control the infection, are briefly described elsewhere.[24] There is also now an apparent correlation with a number of viruses causing chronic infections and their tropism; there seems to be a correlation between the ability of the infectious agent to replicate productively in macrophages/monocytes (and possibly related cells) and the lack of protection by specific antibody *in vivo*, even though it might neutralize viral infectivity *in vitro*.[24] The need to activate infected cells of this lineage by factors such as gamma-interferon or perhaps to cause lysis of the infected cell by the CD8 + T cell may be a major requirement if a vaccine is to be effective.

Vaccines based on peptides from protective antigens seem at this stage to be a likely possibility for some parasitic diseases, such as malaria and perhaps for a few bacterial diseases, such as those caused by Streptococcus where the protective M protein shares an amino acid sequence with a peptide in heart muscle, a case of molecular mimicry resulting in autoimmune disease (see Chapter 1). Peptides and, in general, isolated proteins, are poor at inducing class I MHC-restricted T cell responses. Should such a response be required to control infection, ways will need to be found to achieve this, possibly by varying the method of presentation.

The above examples illustrate the growing need to be able to measure specific T cell responses in the development of some future vaccines. It may well be equally important to be able to measure these responses in the development of vaccines to control human fertility. The release of gamma-interferon may be an important triggering mechanism in the initiation of autoimmune disease;[2] the time may not be far-off when this is one of the parameters measured to judge the likelihood of inducing autoimmune disease following vaccination to control fertility.

Measurement of specific antibody

In the past, measurement of specific antibody during the course of an infection or following vaccination has not presented much difficulty. Techniques such as enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) which offered increased sensitivity, reproducibility and mass performance became widely available and generally were reliable if the level of specific binding of antibody was substantially higher than non-specific binding levels. There may well be occasions, such as the examples given earlier in this chapter, that require substantially greater sensitivity and at least an equal degree of reproducibility as was found necessary in measuring responses to the hCG peptide–diphtheria toxoid vaccine in humans.

Measurement of antibody isotypes, e.g. after immunization at a mucosal surface (IgA, sIgA) or if complement-dependent lysis of infected cells is important (IgG1, IgG3, IgM in humans), is also likely to become increasingly important as more in-depth studies on determining protective responses are carried out, e.g. the requirement for complement fixing antibodies to control staphylococcal mastitis in ruminants.[25]

Generation of immunological memory

It has been proposed,[20] and should be self-evident, that long-lived protection from disease after vaccination may critically depend upon:

1. the generation of a large pool of B and T 'memory' lymphocytes; and
2. the persistence of specific antigen (or its equivalent, such as an idiotype/anti-idiotype network). It would at least be of considerable interest to combine such measurements with ongoing studies of some aspects of antigen presentation, such as microcapsules etc. in controlled release formulations. If the antigen in use can be tagged in some way, such as labelling with ^{125}-iodine and protected from degradation while encapsulated, such an approach should allow a relationship to be established between antigen persistence, memory cell formation and persistence, and the time for which antibody is produced (or antibody-secreting cells are found in a target organ). Such information must be of value in the design of a vaccine.

Autoimmune reactions following vaccination with existing vaccines

Most vaccines in common use today, and a number of vaccines under test, are composed of whole organisms, viruses or bacteria, which are either infectious or inactivated. Most have a very good safety record and many have been administered (e.g. those in the Expanded Programme of Immunization) to many millions of recipients, beginning many years ago. None of these vaccines is known to cause autoimmune disease; it would be very surprising, in view of the already extensive evidence for molecular mimicry and the complexity of the intact organisms, if vaccination with these preparations did not induce some autoimmune reactions. In view of the natural concern about autoimmune reactivity after administration of a self antigen, it would be of great interest to see whether antibody responses induced by administration of, say, two or three traditional vaccines (using pre- and post-vaccination sera) caused reactivity with sections from a panel of tissues similar to that used to monitor vaccination by fertility-control vaccines. An investigation of this nature, if it revealed cross-reactivity between vaccine strain organisms and a number of tissues, might lead to delineation of the specificity of reactions which may not

be associated with disease. It would also further the case for using markers of T cell activation, perhaps initially in conjunction with autoantibody formation, as an indication of the likelihood of induction of autoimmune disease. It may also help to identify alternative immunogens capable of eliciting effective immunity without such cross-reactivity.

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11 Quality assurance of vaccines

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Introduction

The quality assurance of vaccines can be defined as the sum of precautions and measures which provide confidence that vaccines which are used are safe and effective. The prime responsibility for the safety and efficacy of a product is carried by the manufacturer. The manufacturer's quality assurance department has to monitor the activities of the production and quality control facilities. At the national level, if quality assurance is to be more than a pious hope, some kind of regulatory structures such as National Control Authorities (NCA) and National Control Laboratories (NCL) must be established. Quality assurance of vaccines constitutes one aspect of consumers' general protection.

This chapter addresses quality assurance at the national level and does not describe the detailed activities of a manufacturer's quality assurance department in relation to specific products.

Although there is general agreement that the quality of vaccines should be the same for all users irrespective of their resources, it is a matter of fact that the modalities by which countries ensure the quality of a biological product vary with a number of factors such as tradition, experience and resources. More logically, they also vary with types of vaccines (controls for live vaccines differ from those for inactivated vaccines) as well as with methods of production (e.g. controls of rabies vaccine manufactured from brain tissue are not the same as those for vaccines derived from primary cells, which themselves differ from those for vaccines derived from continuous cell lines).

Differences in procedures by which countries ensure the quality of biological products are unavoidable; however, irrespective of countries and products, certain issues must be addressed, and a comprehensive quality assurance system should include several steps which are reviewed later in this chapter.

Manufacturing authorization

The authorization by the NCA of a country for the manufacture of biological products should be given only after assurance has been gained that manufacturing and quality control will follow rules commonly called Good Manufacturing Practice (GMP), and will be made according to the WHO Requirements for Manufacturing Establishments and Control Laboratories.[1]

The above-mentioned documents contain certain requirements or sets of rules, such as the following.

> The availability of basic commodities (water, gas, electricity, steam).
> The availability of adequate facilities, e.g. for waste disposal.
> The existence of adequate and separate structures for production and for control; for production, it may be necessary to have facilities designed for preventing the spread of specially hazardous agents such as sporulated bacteria (see the classification of infective micro-organisms by risk groups into four categories in the WHO publication *Laboratory Biosafety Manual*);[2] in the case where several live viral vaccines are produced simultaneously in the same facility, separation of the manufacturing processes must be achieved. If the same facility is used at different times for producing different live attenuated vaccines, proper disinfection should be carried out before the production of a new vaccine is initiated.
> The use of equipment which is properly maintained and which can be sterilized.
> The control by appropriate physicochemical tests of all raw materials.
> The manufacturing of products by staff in good health, respecting the rules of hygiene, having proper training and qualification, who are adequately supervised, to whom responsibilities have been clearly assigned, having at their disposal written instructions detailing modes of operation, recording all relevant observations and results of tests in registers with numbered pages.
> The storage at the recommended temperature until after the end of the expiry date of a certain number of vials or ampoules from all batches of vaccines which are supplied, so that further tests can be undertaken whenever necessary.

The rationale for requesting the meeting of certain conditions for the granting of a manufacturing authorization is that products manufactured according to GMP rules are more likely to be of good quality.

Assurance that GMP, WHO and national rules are observed can be gained by inspection, and such inspections need to be renewed from time to time, e.g. every 2 years; they should be carried out during working hours, with or without notice. These visits result in the issuing, renewal or withdrawal of authorization to manufacture and this obviously implies that 'authorized' manufacturers are registered nationally.

In the case of imported products, the assurance that GMP rules are followed is gained either through certification by the NCA of the country of production, or, if it can be arranged, through inspection organized by the would-be importer. In all cases, any important changes in the registered procedures for manufacturing and control must be reported by the manufacturer to appropriate bodies.

Licensing

Licensing involves a decision to allow the use of a product subsequent to its successful evaluation. It is usually granted for a given period of time and is subject to renewal. It takes place after a series of steps, which should include the following.

Review of manufacturer's data on production methods and laboratory tests

The following list, which is not necessarily complete, has been prepared by distinguishing between data which need to be obtained at least once or, on the contrary, for every production lot.

Data which need not be generated for each production lot

Characterization of banks of seeds

The history and result of characterization of banks of seeds (cells, bacteria, viruses) should be made available to licensing authorities. This includes information on the origin of the isolate, whether cloning was carried out, number of passages etc. Cell banks are expected to be characterized as regards species markers (e.g. isoenzymes); karyology (chromosome mapping, euploidy or aneuploidy status); studies of tumorigenic potential are also of interest, although less so if the cell line is known to be tumorigenic, in which case reliance for safety will be placed on the maximum permitted amount of cellular DNA remaining after purification (see Table 11.1).

Cell and virus banks need also to be characterized as regards extraneous agents which might have been present from the very beginning, introduced subsequently through contaminated serum used as growth factor, or through unsatisfactory laboratory operations. Whereas tests for extraneous bacteria and fungi are simple, those for mycoplasms are less so and those for viral contaminants may be very elaborate and include inoculation of seed stocks into animals of various species and into cell cultures of different origins; in the case of virus seeds, this requires the addition of suitable antisera for neutralizing the vaccine virus. Further assurance on safety related to the absence of contamination of inactivated vaccines can be gained by demonstrating the capacity of the manufacturing process to eliminate or inactivate extraneous viruses which have been added intentionally to experimental batches ('spiking experiments'). Detection of non-expressed viruses (e.g. endogenous viruses such as retroviruses) or viral sequences integrated into the genome of cells may require special techniques such as, respectively, inoculating cell cultures from other species and/or using DNA probes. Importantly, cell banks which have been found not to contain extraneous agents may have to be tested again if cells have been inserted with plasmids, since such manipulations can result in the expression of viral sequences otherwise not expressed.

Table 11.1. *Examples of in-process tests for controlling the safety and efficacy of bacterial and viral vaccines prior to their filling into final containers*

Type of test	Type of vaccine		
	Replicative	Non replicative	
	(live attenuated)	Inactivated (fractionated and non-fractionated)	Recombinant
Absence of contamination	+	+	+
Characterization of cell substrate[1]	+	(+)	+
Characterization of the vector[2]	−	−	+
Absence of extraneous viruses[3]	+	(+)	(+)
Confirmation of inactivation	−	+	−
Confirmation of number of culturable particles or required activity	+	+	+
Amount of total protein	−	+	+
Absence of blood group substances	−	(+)	−
Amount of residual animal serum[4]	(+)	(+)	(+)
Potentially hazardous chemical reagents	−	+	−
Residual contaminating cellular DNA[5]	(+)	(+)	(+)
Purity[6]	−	+	+
Molecular size of antigen	−	+	+
Genetic marker studies	+	−	−

Note:
+ = test required.
(+) = test required for some products.
− = test normally not required.
[1] If cell culture used for production.
[2] For example studies of gene insert by nucleotide sequencing or restriction enzyme.
[3] If virus seed and/or cell substrate used for production.
[4] If serum used as growth factor.
[5] If continuous cell line used.
[6] Using some of the following: high-pressure liquid chromatography (HPLC); sodium dodecyl sulphate (SDS); polyacrylamide gel electrophoresis (PAGE); Western blot; single immune radial diffusion (SRD); immune electrophoresis (IEOP); enzyme-linked immunosorbent assay (ELISA); etc.

Finally, information on the conditions of storage of seed banks should be provided.

The advantages of using banks of seeds are well known: they reside essentially in the manufacturing of consistent lots, which is a fundamental aim of biological standardization; risks of antigenic and pathogenic drifts are minimized; and the amount of testing (e.g. for extraneous agents) can be reduced.

Details on production methods

Details of production and control methods are expected to be provided by manufacturers in a document listing standard operating procedures (SOP)

and limits for acceptance (see later). For example, for recombinant DNA products, appropriate details on methods of construction of DNA sequence and insertion of plasmids should be given. The amino acid composition of the intended product, e.g. hepatitis B surface antigen (HBsAg), should be provided as well as its (at least partial) amino acid sequence. The sequence identity of the recombinant to the natural product does not necessarily guarantee that the recombinant product will be protective, especially since minor changes in spatial configuration of the antigen molecule (tertiary structure changes) may result in profound immunogenic differences.

In principle innocuity of the product

Assurance of the 'in principle' innocuity of the product implies addressing certain issues such as the following.

1. *Oncogenicity*. In view of their growth characteristics, and also of the fact that certain types of products (e.g. monoclonal antibodies) can only be manufactured from malignant cells, tumorigenic cell lines are increasingly used for the production of biological substances, including vaccines.[3] The main conclusion of a study group which met in WHO, Geneva, in 1986[4] was that such cells are acceptable, provided the amount of residual cellular DNA in each single human dose of the final product is not more than 100 picogrammes. This conclusion was reached on the assumption that the purification process does not result in the concentration of certain oncogenes or groups of oncogenes. The group, however, agreed that requirements for minimal amounts of residual cellular DNA were irrelevant in the case of vaccines administered *per os*.
2. *Teratogenicity*. Certain live viral vaccines when administered to pregnant women might conceivably cause malformations to fetuses. Although there is no evidence of this occurring, even with rubella vaccines, the recommended policy is that unless the absence of teratogenicity has been demonstrated, live vaccines should not be used in pregnant women.
3. *Virulence*. Seed viruses for the manufacture of certain live attenuated vaccines such as yellow fever, rubella, varicella, measles and mumps need to be tested for neurovirulence in monkeys (see corresponding WHO Requirements).
4. *Time validity*. Assurance that at the end of the expiry date products still possess the minimum 'potency' required should be given, e.g. by determining the degradation kinetics, in order to establish the minimum amount of product to be introduced in final containers at the time of filling; in addition, it is advisable to carry out at the end of the expiry date potency tests on several batches of vaccines kept under the recommended storage temperatures.

5. *Other information.* The file which is submitted for licensing should contain other information enabling the relevant body to ascertain that no ingredients, such as beta lactam antibiotics, normal human serum, human serum albumin other than heated 10 hours at 56°C, or culture medium containing blood group substances or proteins not completely digested, are used for manufacturing. The file should also contain all relevant information on the type of final container, the label, the package, the instruction note to users, which should indicate the formulation of the product, the indications and contraindications, the recommended schedule of administration, the side reactions, the conditions of storage, etc.

Review of results of tests which need to be performed on every production batch

Whereas certain tests need to be carried out only once or on a limited number of occasions, it is customary to demand that other tests be implemented on every production batch. Modalities for such tests as well as details of production methods should be provided under the form of the SOP which, for obvious reasons, is usually confidential in nature.

For the proper control of biological substances including vaccines, tests are carried out not only on final products but also at various stages of manufacturing. The reason for such 'in process tests' is that it may be difficult or even impossible to assess the quality of vaccines by testing performed solely on final products; for example, the 'irreversibility test' for diphtheria and tetanus toxoids needs to be done on purified materials, not on final *adsorbed* products; the testing for neurovirulence of oral poliomyelitis vaccine (OPV) must be done on *monovalent* bulks. Tests which need to be performed on each batch are carried out at various stages of manufacture.

At the bulk level

The following are examples of tests performed at this stage.

(a) Tests for the absence of virulent mycobacteria (BCG).
(b) Tests for complete inactivation of live particles, such as those for inactivated viral vaccines (rabies, poliomyelitis).
(c) Tests for the absence of specific toxicity for diphtheria and tetanus toxoids.
(d) Tests for irreversibility, e.g. on purified diphtheria and tetanus toxoids.
(e) Tests for neurovirulence (OPV).
(f) Tests for antigenicity in which the antigenic content is determined (e.g. Lf content of tetanus and diphtheria vaccines).

At the final bulk and/or at the final product level

Examples of tests performed at this stage are as follows.

(a) Tests for potency on inactivated vaccines by which one ensures that products injected into animals elicit antibody production or induce protection at least equal to that obtained with products which have been found to be protective in humans. Such tests are of major importance since they are intended to predict that the protective properties of products will be satisfactory in target species. Whenever applicable, a reference material properly calibrated against the relevant WHO Standard[5] should be included in tests for potency so that potencies of test products are expressed in relation to that of the reference. This has two important advantages: (1) minimizing the effect of variations of conditions of assay; such conditions may be difficult to control and random variations, if not taken into consideration, could lead to false conclusions; (2) allowing the worldwide comparison of products of different origins since, for given products, activities are expressed in the same unitage.

Much emphasis should be placed on potency tests and the way they are performed. Proficiency studies, usually carried out at an international level, are most useful in this respect.

(b) Test for purity: such tests are aimed at verifying that possible impurities such as serum proteins, agents for inactivation and preservatives do not exceed authorized limits.

At the final product level (Table 11.2)

Examples of tests performed at this stage are as follows.

(a) Tests for the absence of contamination: (such tests are also performed throughout the production process).

(b) Tests for potencies of live vaccines: potencies are controlled by determining the number of culturable particles present in final containers to ensure that numbers per single human dose are not less that those which have been found to induce satisfactory protection and/or seroconversion in humans.

(c) Tests for adjuvant content.

(d) Tests for absence of general toxicity: such tests, which are usually carried out on mice and guinea-pigs, are aimed at verifying that no hazardous substances have been introduced into containers prior to their sealing.

(e) Tests for identity: such tests (usually on one or two final containers picked at random) are made in order to verify that the product is the one indicated on the label.

(f) Tests for thermal stability: such tests are usually required for live vaccines (BCG, yellow fever), which, unless properly manufactured and stabilized, might rapidly become non-potent should the recommended conditions of storage not be maintained, as sometimes happens in tropical countries.

Table 11.2. *Summary of tests to assure the safety and efficacy of bacterial and viral vaccines in final containers*

Type of test	Type of vaccine		
	Replicative	Non replicative	
	(live attenuated)	Inactivated (fractionated and non-fractionated)	Recombinant
Absence of contamination	+	+	+
Innocuity	+	+	+
Amount of preservative	−	+	+
Amount of additive or adjuvant	(+)	+	+
Absence of pyrogens and/or endotoxins	−	(+)	(+)
Residual moisture content (if freeze dried)	+	+	+
Total protein	−	(+)	+
pH (if liquid)	(+)	+	+
Identity	+	+	+
Potency or number of culturable particles	+	+	+
Stability (thermal)	+	−	−
Appearance	+	+	+

Note:
+ = test required.
(+) = test required for some products.
− = test normally not required.

(g) Tests on certain physicochemical characteristics, such as tests for pH, or, in the case of lyophilized products, tests for residual moisture or, if applicable, tests for vacuum; such parameters may affect the time stability of products.

(h) Visual examination of each final container, in order to detect any gross abnormality, such as improper filling or sealing or stoppering etc.

Although concern for safety and efficacy should be the same for all vaccines, control modalities cannot obviously be the same for different types of vaccines such as oral polio and tetanus vaccines. In the process of defining appropriate tests, one should therefore operate on a case by case basis and exert commonsense. Table 11.3 gives examples of tests which should be considered prior to licensing and/or releasing antifertility vaccines. For many vaccines, International Requirements exist which can be adopted by countries, partly or in total. The list of such requirements as well as of corresponding revisions and addenda is published once a year as an annex to the WHO Technical Report series of annual WHO Expert Committees on Biological Standardization.[6] The WHO Requirements include model summary protocols which can be used by producers to report the way in which products are manufactured and

Table 11.3. *Examples of tests which should be considered prior to licensing and/or releasing antifertility vaccines*

I	Vaccine produced by rDNA technology	
	Cell bank	If mammalian cells used: absence of contamination by extraneous agents
		Genotype and phenotype remain unaltered on recovery
	Single harvests	Absence of pyrogens
		Yield within accepted limits
II	Vaccines derived from synthetic peptides	
	Synthetic peptide	Correctness of sequence and tertiary configuration
	Carrier protein	If toxoid is used: absence of reversibility and specific toxicity
	Peptide–carrier conjugate	Peptide: carrier ratio
		Peptide remains unaltered
III	Vaccines produced by rDNA and synthetic peptide technologies	
	Purified bulk	Identity and purity – PAGE, HPLC, peptide mapping, amino acid composition, amino acid partial sequence
	Final bulk	If adjuvant is used, verify concentration and adsorption and coupling are correct
		If microcapsules are used, verify number and sizes of capsules and amount of antigen per capsule are correct

Note:
PAGE = polyacrylamide gel electrophoresis; HPLC = high-pressure liquid chromatography.

tested. During the licensing process by NCAs, the reviewing of summary protocols of, for example, five consecutive batches of the product is helpful for ascertaining the degree of consistency. Consistency of production and control is a good indicator of the capacity of manufacturers to produce, repeatedly, lots having similar characteristics. In case of doubt, NCAs may wish to have certain tests repeated by independent laboratories.

The obvious corollary of the necessity of maintaining consistency of production is that control authorities should be informed of any important change in manufacturing and controlling processes.

Results of clinical studies

Requests for licensing should be accompanied by published and non-published results of clinical studies[3] establishing the safety and efficacy of the product. Such studies should involve an adequate number of subjects representative of target groups as regards sex, age and, if appropriate, immunological status. In the case of a live vaccine, it may be appropriate to determine the safety of the product not only for vaccinees, but also for contacts and, if applicable, for other species. Studies on efficacy should determine the duration of protection.

Appropriateness

In the case of a would-be imported vaccine, it may be useful before addressing issues such as production lot data and clinical studies, to examine the cost of the product, its reported efficacy, the resources which are available for purchase and administration, and the incidence and severity of the disease.

Other data

It may be of interest to NCAs of importing countries considering the granting of a license to require from the producer and/or the NCA of the exporting country information on matters such as:

the list of countries in which the vaccine has been licensed;
the number of doses and batches, if any, rejected by the NCA of the exporting country, e.g. during the last 3 years;
the number and nature of serious side reactions per number of doses administered, and the system used for collecting such data.

Consequences ensuing from the granting of a license

The granting of a license requires the following issues to be addressed by the NCA.

Defining release modalities

The release of vaccine is an operation to authorize the marketing of a *given batch* of a product for which a license has been granted. If applicable, the decision to release a batch should be made after having ensured that the batch has been properly manufactured and controlled; one way to do this is to examine the relevant 'summary protocol'. Tables 11.1 and 11.2 provide lists of tests, some of which are obligatory for all vaccines and others which are appropriate for certain specific products. Those tests which are obligatory must provide evidence that the specifications of the product are not inferior to those described in the SOP, and that appropriate limits for parameters such as purity and potency are not exceeded. Additional testing may be required from producers by the NCA or NCLs if considered appropriate. The degree to which certain tests should be repeated by the NCL is a matter for such laboratories to decide. In cases where consistency of production and testing has been established, there may be little justification for having tests repeated by NCLs. Regardless of whether tests are executed independently from manufacturers, an important aspect has to do with the way in which sampling is done.

Release modalities vary with countries, and one of the following three systems is generally used.

Free release: licensed manufacturers are free to release batches without providing further information to the NCA or NCL.

Partial free release: licensed manufacturers must provide information to NCA or NCL for each batch which is put on the market, but need not obtain a release certificate.

Release after the NCA or NCL has issued a release certificate: it is this system which is recommended by the WHO for biological products to be exported.

Post-marketing surveillance system

A post-marketing surveillance system is desirable to ensure that nothing unexpected occurs after the administration of a product.

The reason for setting up a surveillance system is that there is always a possibility that certain defects have escaped laboratory controls. If post-marketing surveillance suggests that this has occurred, it is important to recall, at an early stage, all vials or ampoules from the relevant batch which have been distributed. Another role of a surveillance system is to monitor the rare untoward reactions which cannot be detected by current tests, a process which is made easier if an adequate reporting system exists.

The post-marketing surveillance should also bear on efficacy, and this can be done by following epidemiological data and undertaking appropriate serological studies.

Exercising surveillance after vaccines have been released may reveal problems, such as lack of efficacy or occurrence of local and/or systemic reactions not necessarily related to improper manufacturing and testing, but caused, for example, by poor storage, use of non-sterile syringes or other factors outside the control of the producer. Post-marketing surveillance meets with specific problems, which as far as drugs are concerned have been examined in 1985 by a working group.[7] It requires the collaboration of several parties including manufacturers, physicians and NCAs. In the USA, there is a 'Stimulated Passive Monitoring System' by which persons having received vaccines purchased from public funds are invited to report adverse reactions occurring within 4 weeks of administration.[8] A project aimed at co-ordinating and improving the international reporting of post-marketing adverse drug reactions among a limited number of countries has been recently initiated by the CIOMS.[9]

Conclusion

Quality assurance for vaccines has many similarities with quality assurance for chemically defined pharmaceutical products. However, unlike pharmaceutical products, biological products cannot be tested solely by physicochemical methods and their testing often requires the use of appreciable numbers of laboratory animals and cell culture systems, which increases the cost of

production. Furthermore, it is often not possible to confirm the safety and efficacy of a product by tests performed solely on the final material. The problem becomes even more complicated if products are exported to countries having requirements differing from those of the exporting countries. This is why WHO believes it important that WHO member states adopt and adhere to International Requirements for Biological Substances.

The assurance for quality has many facets, the chief of which include the quality of the manufacturing process, the correct implementation of appropriate laboratory tests, the licensing and monitoring by the national authorities, and the post-marketing surveillance.

In the process of providing quality assurance, it is desirable to achieve a reasonable balance between costs related to the amount of testing and benefits which are expected from the tests. Excessive testing might result in products being made available at unaffordable prices, and could even prevent the availability of products. A classical example is the assurance for the absence of contamination which, in order to be complete, would require the testing of every final container.

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12 Regulatory aspects of vaccine development

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Introduction

The regulatory aspects of vaccine licensing include all the steps from the beginning of the development until the last dose is administered without adverse results. The regulations governing biologics must be considered from the initial design of the vaccine, the selection of strains, cell cultures, plasmids, etc., through laboratory tests, clinical trials and manufacturing to the final distribution method of the labelled and packaged product.

In any organization, those responsible for the development, production and distribution of a vaccine must take into consideration the legal, ethical and social aspects of a vaccination programme. These factors form a significant part of the negotiations with the regulatory authorities, whenever a novel approach is taken.

The government bureaux regulating pharmaceuticals and biologicals in any country generally form part of the ministries or departments of health and are charged with the responsibility of ensuring that the public receives the safest and most efficacious products available. 'Consumerism' is today not just a movement in the developed countries based on probings into unethical practices of industrial giants, but a worldwide demand by the general public to receive state-of-the-art value for their expenses, especially with respect to health care.

Therefore, although the legal, ethical and social aspects of antifertility vaccine development and use are discussed in detail elsewhere (see Chapters 13 and 14), they cannot be excluded from a discussion of the regulatory considerations.

Vaccines are generally administered as prophylactics to normal, healthy persons in order to protect them against infectious diseases. An antifertility vaccine will also be used by normal, healthy adults, but is specifically intended for the fertile age group for protection against an unwanted pregnancy. Unlike its more conventional counterparts, therefore, such a vaccine is not intended to confer herd immunity or to provide a protective barrier against a communicable disease, but is to be considered an addition to the range of birth control methods available to the users and providers of health services.

Although there are recognized risks to any vaccine and drug, and although the currently approved 'birth control pills' carry warnings of potential serious long-term effects, every effort must be made to ensure that an antifertility vaccine is devoid of significant hazards.

While the major impact of an antifertility vaccine is likely to be in the developing countries, its development and manufacturing will probably take place in one or more of the developed countries, at least initially. It is essential to ensure that such a vaccine fulfils all regulatory and ethical requirements but it is equally essential that this is seen and understood to be the case by the user population.

The first question that needs to be asked is: 'Is this vaccine safe, effective and acceptable, in every respect, to the majority of the developed countries?' Obviously, if the answer is 'no', it will be both extremely difficult, and also immoral, to promote its use in a developing country. Therefore, an antifertility vaccine should be such that anyone in the developed countries should be able to consider this product as a safe alternative to, for example, long-acting injectable steroid contraceptives or to vasectomy or tubal ligation.

Another question that is certain to be raised by many potential user groups concerns the mechanism of action of the vaccine and whether it will be perceived as a contraceptive or an abortifacient. This and other issues are discussed elsewhere (see Chapter 14).

For these reasons it is essential that a successful antifertility vaccine is both licensable and promotable in any developed and developing country.

Pharmacopoeial requirements

The major vaccine-producing countries can be divided into these pharmacopoeial categories.

Australia and New Zealand

Follow the British Pharmacopoeia (BP), but also consider WHO and the US Code of Federal Regulations (CFR) as appropriate.

European Economic Community (EEC)

All EEC countries must comply with the European Pharmacopoeia (EP), although many of them also have national pharmacopoeias such as:

BP in the United Kingdom;
Deutche Arzneibuch in the German Federal Republic;
and many others.

In general, where there is a difference, the EP takes precedence. These countries mostly also comply with WHO requirements for biologicals.

United States of America

Follows the Code of Federal Regulations, specifically Title 21 (21 CFR) with reference to the United States Pharmacopoeia (USP).

Canada

Complies with Canadian Food and Drugs Act and the Food and Drug Regulations in general, but considers, also CFR and WHO as appropriate.

USSR

USSR Pharmacopoeia basically follows WHO for vaccines.

Japan

Follows Minimum Requirements for Biological Products.

People's Republic of China

Follows WHO, but is working on its own regulations also.

Most other countries

Follow WHO, occasionally with some national specifications for certain products.

Licensing of a vaccine

While, in general, drugs and other pharmaceuticals are subject to approval by the national control authority, a vaccine is subject to licensing. Implied in this term is the permission to produce the vaccine by a proven and approved method, in approved facilities, by approved personnel. These issues are discussed in detail elsewhere (see Chapter 11) but are summarized here for illustration.

Vaccines are generally subject to lot-by-lot release by the national control authorities after they have reviewed the production and testing records and tested (or at least been given the opportunity to test) a representative sample of the lot submitted for release.

The road to licensure is a difficult path crisscrossed by mine fields, booby traps and side tracks often leading nowhere. If this sounds pessimistic, consult some of those who have been charged with the responsibility of guiding the brilliant idea of a scientist through to a licensed, saleable vaccine.

And then, when the requirements of the licensing authority are satisfied, there are the lawyers to contend with. Beginning with a consumer concern for adverse reactions, the liability issue has developed into an insidious 'litigation virus'. This was first 'isolated' in the United States, but is now spreading rapidly throughout the world, and although vaccines have been developed under government indemnification schemes or no-fault insurance, the only 'cure' today still appears to be an unlimited supply of money.

Physicians, nurses, manufacturers, hospitals, health units as well as regulatory authorities are all targets for law suits and may have to spend much time and money on court cases dealing with the occasional severe adverse reaction. While nobody denies the terrible tragedy to the individual family stricken with such an incident, it would be a greater tragedy if litigation leads to the disuse of life-saving vaccines.

The following is an attempt to map out the major steps along the road to licensing, including some of the hazards one encounters along the route. Basically the route and the hazards are the same in most countries which have a regulatory bureau or organization dedicated to the control of biologicals. Most of the differences are in the format of the submissions, the time it takes to review them, the specific forms used to introduce the subject and, sometimes, the degree of willingness to accept any deviation from the regulatory requirements, even when it is amply justified as an improvement. Some of the more major differences will be pointed out.

The path to licensing generally follows this course.

Laboratory development and testing.
Establishment Licence application.
Preparation of pilot lots/clinical lots.
Request for permission to conduct clinical trials:
North America: Investigational New Drug Submission (IND)
UK: Clinical Trial Certificate (CTC) or, under special circumstances, Clinical Trial Exemption (CTX).
Clinical trials.
Product Licence application.
Licensing.

Laboratory development and testing

It is assumed that, at this stage, the vaccine is conceptually developed, the antigen is identified and characterized, the basic research completed, and the method of antigen production determined – whether by classical fermentation methods or by recombinant DNA (rDNA) technology; basic concepts regarding media, substrates, target population, route of administration and potential toxicity and reactogenicity are thought out and properly recorded; also, that the tests for safety and potency and other important parameters have been selected, validated and a proper rationale developed.

The next step consists of the laboratory studies undertaken in order to justify the initial (Phase 1) clinical trial. At this stage, Good Laboratory Practices for Nonclinical Laboratory Studies (GLP),[1] becomes the guiding concept.

While this set of rules is referred to here by the terminology from the US Code of Federal Regulations, the concepts are basically the same in many other countries, although the requirements are not expressed as definitively.

According to GLP the laboratory must:

appoint a study director who has overall responsibility for every aspect of the study, and who must ensure the absence of any bias;
prepare a study protocol that clearly sets out the objectives and methods for all in-vivo and in-vitro testing, experimental design and statistical methods, especially as it relates to the safety of the product; the choice of tests will be influenced by, but not restricted to, requirements and guidelines issued by regulatory agencies;
be willing to accept inspection by a representative of the regulatory authority which will be requested to allow the trial; this is especially true for the United States Food and Drug Administration (USFDA).

Establishment Licence application

An Establishment Licence for biologicals is generally based on an overall licence for the manufacturing facilities in which the product will be made. This licence is subject to a series of amendments or addenda when the facilities are changed in order to adapt them to new products or is modernized to meet new competitive standards or regulatory requirements. While an Establishment Licence is not required for a laboratory to be permitted to commence a clinical trial, it is essential to have a clear concept of the facility in which the product lots for clinical trials will be manufactured. There should not be any significant changes, which may change the critical parameters of the product, between the time of preparation of the clinical trial lots and the time of preparation of the lots in support of final product licensing. Any manufacturer without an approved establishment would be wise to discuss with members of the appropriate regulatory body the structure of facilities and equipment to be used, and to seek, at the earliest possible time, official approval of their establishment.

While the requirements may vary somewhat between countries, the basic information essential for the regulatory approval of a laboratory/plant for vaccine manufacturing are:

location;
buildings (production, quality control, animal housing, raw material storage, etc.);
structures (walls, ceilings, floors, etc.);
lay out;
air handling (class of air, filters, pressure differentials);
flow of material (clean, dirty, containment procedures, sterilizing facilities etc.);
flow of staff (entry, exit, dress rules, access restrictions);
water supply (tap and distilled – quality and testing);
steam supply;

sewage handling;
incubators, refrigerators and freezers (monitoring);
quarantine facilities;
filling and packaging facilities (container specifications, sterilization and handling);
equipment and process validation methods;
organizational charts;
curricula vitae of responsible personnel;
environmental impact of facility.

While all these items are essential, it is worthwhile noting the rapidly growing emphasis on validation. In principle, this is the act of demonstrating that any process, procedure, test, piece of equipment, etc., functions as intended and designed and that it gives the expected results.

When an establishment is approved, it becomes easier to amend it or add to it in order to include or alter the facility intended for the preparation of the new vaccine. In the USA an actual Establishment Licence is not issued until the establishment has a product ready for licensing. Similarly, any amendment to an Establishment Licence is tied in with the licence or amendment to the product. It is, however, prudent to have an *a priori* review of the intended facility changes with the authorities before making a major capital investment.

It is essential, regardless of the initial country of licensing, that a potential manufacturer becomes familiar with the basic Current Good Manufacturing Practices (CGMP).

This set of commonsense rules for the manufacturing of drugs and biologicals for human use applies fairly universally with only minor differences. Anyone complying with the US CGMP[2,3] and the WHO Good Practices in the Manufacture and Quality Control of Drugs (unpublished WHO document: WHO/PHARM/82.4, Rev. 3) would probably be acceptable, more or less universally. The 'more or less' is inserted as a precaution, since individual inspectors from some countries may interpret the CGMP differently.

As implied in the title of these regulations (current), they essentially refer to state-of-the-art and are subject to updates based on innovations in the field relating to quality control, qualifications of personnel, structure of buildings, sterilizing facilities, air handling etc. Any significant improvement in one of the areas related to CGMP introduced by a member of the biological (or indeed pharmaceutical) industry may eventually become the industry standard.

Preparation of pilot lots/clinical lots

As the word implies, the pilot lots are often made in a pilot plant which is separate from the production plant and where the lots are prepared on a

smaller scale, often with some adjustments in procedure from lot to lot. This is a commendable procedure as long as the lots produced are intended only for experimental purposes. If one or a series of pilot lots become earmarked for a clinical trial, even the first small Phase 1 safety study, the concept changes. In this case the pilot plant must meet the CGMP and subsequent lots prepared for the Phase 2 and Phase 3 trials must be prepared in the same way as the first lot. Only very minor changes in processes and procedures are permissible and these must be clearly explained and justified in the submission to the regulatory authorities (see next section).

It is therefore essential that substantial developmental work is performed as early as possible to determine the requirements for a scale-up to an economically viable batch size.

It is not unusual for a manufacturing process to fall apart during the transfer from pilot scale to full scale. This is especially true for the biologics industry where, for instance, the move from a 10-litre to a 1000-litre fermenter can require a significant change in a number of parameters such as pH, cell density, stirring rate, oxygen supply, growth curve, etc. These changes may in turn raise serious questions about potential changes in the end-product, with resultant requirements for additional safety tests and a new Phase 1 trial.

If these questions are not anticipated in advance, the cost in both time and money can be substantial and discouraging, if not totally destructive to the project.

The permission to conduct clinical trials

The requirements for a clinical trial permit differ from country to country. In the United States, before a clinical trial of a vaccine can proceed, an Investigational New Drug Submission (IND)[4] must be filed with the Center for Biologics Evaluation and Research (CBER) of the FDA.* In Canada, a similar application goes to the Bureau of Biologics (BOB) of the Health Protection Branch, and in the United Kingdom the Department of Health and Social Services (DHSS) is requested to issue a Clinical Trial Certificate (CTC). Variations of these systems apply in most countries.

It is important for a clinician or clinical trial sponsor to approach the appropriate authorities, generally a department within the ministry of health, at a very early stage in the planning exercise and before significant sums of money are committed to the investigation in any country.

In the following, the term IND will be used to cover any government authorization to proceed with a general trial in any country. It is important to be aware that for clinical trial data to be applicable in some countries, such as

* While the CBER of the USFDA is responsible for vaccines and other biologicals in general, antifertility vaccines are controlled by the Division of Metabolism and Endocrine Products, Center for Drug Evaluation and Research, USFDA.

the USA, the trial must have been conducted basically in compliance with that country's IND requirements.

The essential parts of an IND are described below, using basically the terms from the US CFR. Similar data are generally required by most authorities, but the formats and details vary significantly and must be ascertained before a submission is made.

Cover sheet

The 'cover sheet' is the prescribed form generally required as the opening statement of an IND. It includes the main facts regarding:

name, address and telephone number of the trial sponsor (generally the manufacturer);
trial phase (1, 2 or 3 etc.);
commitments regarding:
  start of trial only when IND is in effect;
  institutional review board;*
  conduct of trial in accordance with regulations;
name and title of trial monitor;
name(s) and title(s) of persons responsible for review and evaluation of data relevant to safety of product;
signature of sponsor or authorized representative.

Table of contents

Introductory statement and general investigational plan

Name of product, pharmacological class (e.g. vaccine), active ingredients, route of administration, objectives for trial and planned duration.

Brief summary of previous human experience, reference to other INDs or experience in other countries if available.

Any information regarding the withdrawal of such or a similar product from the market in any country if withdrawal was related to safety and efficacy.

Description (brief) of the plan of investigation including:
  rationale for product;
  indication;
  approach to evaluation of product;
  kinds of clinical trials during first year;
  estimated number of trial subjects;
  any anticipated risk to subjects;
  copy of Informed Consent form for trial subjects.

* The institutional review board (IRB) is, in terms of the US regulations, a board or committee designated by an institution (any public or private entity or agency) to review and approve the initiation of a clinical trial. The major responsibility of the IRB is to assure the protection of the rights and welfare of the trial subjects.

Investigator's brochure

This should contain:

- brief description of product;
- summary of pharmacological and toxicological data in animals (and in humans, if known);
- data on pharmacokinetics, if available (animals and humans);
- summary of safety and efficacy data;
- description of potential risks, side-effects as well as precautions, warnings and special monitoring required.

Protocols

A study protocol must be included for each clinical trial planned. This may be less detailed for the Phase 1 trial, which is generally limited in number of subjects and in scope, and is basically designed as a safety study. Hence the details and the monitoring refer primarily to safety-related parameters such as adverse reactions. Phase 2 and 3 trial protocols require significantly more detail with respect to the experimental design of the study, and should include results of the previous Phase 1 study.

All protocols must contain:

- objectives and purpose of study;
- name, address and qualifications (*curriculum vitae*) of each investigator and co-investigator;
- name and address of research facilities used;
- name and address of each institutional review board;
- criteria for subject selection and exclusion;
- anticipated number of subjects;
- experimental design, control group, statistical design, observations to be made;
- administration schedule, route and dosage;
- measurements, clinical observations, laboratory tests relative to monitoring the efficacy and safety of the product in humans.

Manufacturing and quality control information

This will include:

- process description, including raw materials, media, cell cultures and, if dealing with a rDNA product, all relevant details regarding the selection of genetic code, plasmid and substrate. (NB: Cell substrates have been the subject of many recent meetings, papers and discussions, both at the national level and at WHO, and the selection of a suitable substrate needs much careful consideration);
- quality control testing procedures, including those applied to raw materials.

Labelling

This must indicate clearly that the vaccine is an investigational new drug.

Environmental impact analysis

This is generally confined to the USA and is generally subject to exemption for conventional biologicals. (NB: Products manufactured using rDNA technology are now subject to significant additional review by other regulatory bodies in several countries.)

Pharmacological and toxicological data

This information is obtained from preclinical studies. (NB: teratogenicity and carcinogenicity studies are generally not done for the 'classical type' vaccines which are normally administered only once or a few times in a lifetime. Such studies are, however, commonly required for products produced by rDNA procedures.)

It should also be anticipated that much stricter requirements will apply to an antifertility vaccine, in view of the target antigen, the fact that it may be administered more frequently than a conventional antimicrobial vaccine and because the vaccinee population consists of women of child-bearing age.

Stability data

It is desirable to at least have sufficient data to indicate that the product is stable under normal conditions of storage for the duration of the administration part of the clinical trial.

Other information

The IND submissions should also contain any other information which the sponsor considers relevant to the trial or which the control authority may request.

The language

The language of the submissions must in general be the language of the target country as far as the cover sheet of the IND is concerned. There are varying rules as to what else must be in that language. Generally, English is acceptable (or in some countries required) for the body of the information. In such cases, clinical and supporting data written in other languages should be submitted with a translation into English.

Amendments or changes to the IND

Any changes to the protocol require approval by the institutional review board, where such exists, and must also be reported to the regulatory authority unless the change is in order to eliminate a hazard to the subjects. In such cases, the change can be made immediately and reported afterwards.

Effective date

The time at which the sponsor may proceed with the trial varies greatly from country to country. In many countries (e.g. the UK) a trial cannot proceed until written permission has been issued. In the USA the trial may proceed 30 days after the FDA has received (and acknowledged receipt of) the IND unless the FDA notifies the sponsor that the trial shall be delayed. In Canada the same system applies except that the period is 60 days. In other countries only a notification is required. As stated earlier, it is essential that the appropriate authorities in the country are contacted before any irrevocable commitments are made. In those countries where the trial can proceed after a prescribed waiting period it is prudent to contact the agency by telephone at the end of the waiting period to be certain that a notice to delay trial has not been sent and lost in transit.

Annual reports

Annual reports are required in some countries with summaries of the progress made in the studies to date.

Clinical trials

The clinical trials will, of course, follow the protocol outlined in the IND. This should be ensured through an effective monitoring system. The monitor must also ensure that the trials are conducted within the rules of the Declaration of Helsinki or within the regulations of the country in which the trial takes place. If the rules of that country differ from the Declaration of Helsinki, and the data are to be applied in a country which requires full compliance, these differences shall be described in detail and it shall be explained how these differences offer greater protection to the trial subjects.

Product licence application

The New Drug Application, which for a pharmaceutical product may lead to an approval by the regulatory body, is known as a Product Licence Application for biologicals which, if acceptable, leads to a Product Licence.

In order to obtain a product licence, the manufacturer must:

complete satisfactory clinical trials;
have an approved manufacturing facility;
have submitted a satisfactory licence application, which includes all the production and testing methods as well as completed, satisfactory manufacturing and testing protocols (and certificates of analysis) for at least three, generally five, consecutive lots; the application must also contain all the relevant equipment and process validation

mentioned above under Establishment Licence Application;
have completed a satisfactory pre-licensing inspection from the licence granting authority;
have a product available for distribution.

At a very early stage in the development of a vaccine it is wise to review the requirements for a licence application and go over all the details, which will be requested, before a licence is granted.

It is, of course, not possible to cover every potential issue which may be raised, for it is well known that regardless of how exhaustive such a submission is, there will always be additional questions to answer. It is generally useful, desirable and encouraged that the manufacturer meets with the scientists of the agency prior to any major steps in the proceedings. This applies both to the IND and the licence application, and can lead to very significant time savings for both applicant and regulator.

In a number of countries, for example Canada, Federal Republic of Germany, UK and USA, the regulatory bodies dealing with biologicals have scientists on their staff, which generally facilitates discussion.

Prior to meeting with the authorities, it is essential to become familiar with the general thinking related to the subject matter. In addition to the basic science behind the subject there are generally many sources of information which can serve as guidance, such as:

the pharmacopoeias;
the national regulations such as:
  US Code of Federal Regulations;
  Canadian Food and Drug Regulations, etc.;
  Japanese Minimum Requirements, etc.;
WHO Requirements;
Guidelines and Points to Consider issued by WHO, the USFDA and other agencies.

Post-licensing requirements

When the licence is approved, a new set of obligations is added to those imposed while seeking a licence.

Manufacturing

Following the completion of the qualifying lots which have indicated to the authority that the manufacturer is capable of producing a series of consecutive lots which meet all the requirements, it is now incumbent upon the production department to continue producing such lots consecutively without fail. (NB: Consecutive here mainly refers to safety, i.e. an interruption in lot numbers due to a bacterial contamination or other accidental occurrence does not necessarily constitute a break in consistency.)

Even more than the published minimum requirements and regulations for the product, the approved licence application becomes the standard which must be followed. Any deviation from the approved procedure is a potential reason for regulatory action.

This does not mean, however, that progress stops at the time of licensing. There are, during the life of a product, many needs for change from the stated methods. While this is recognized by most, if not all, regulatory bodies, they will insist that the manufacturer:

- ensures that no unauthorized changes take place; and
  ensures that there is an approval system in place to legitimize necessary changes.

In general, the system requires the existence of a quality assurance department (QA) and a validation department. The change, which may be with respect to processing methodology or to raw material, equipment, testing, etc., will be proposed to QA with appropriate rationale and back-up data. QA must have the authority to reject, request more data/better rationale, or pass the change on to the regulatory affairs department (RA) with a recommendation. It is then the responsibility of RA to make a final decision as to whether the issue is within regulations or not, and, if basically acceptable, falls under one of three categories:

- no regulatory notification required;
  notification of regulatory authorities required;
  cannot proceed till permission from regulatory authorities obtained.

If a procedure or equipment change is introduced, it cannot be implemented until validated by the validation department.

Quality control

In addition to the responsibility of performing (or ensuring performance) of all applicable and required tests, it is common that the quality control department (QC) ensures that the final product protocol (the summary of production records and test results) is properly compiled and, together with test samples, forwarded to the national control authorities (NCA) from which release is required. (NB: Some NCAs do not require detailed protocols and do not perform duplicate testing, and if the product is imported, they rely on the release from the NCA of the country of origin.)

When the NCA has approved the lot or batch, it is the responsibility of QC to issue final release of the lot, and to ensure that the documentation and the release in every respect refer to the actual product.

QC must also, without delay, notify production, QA and RA of unacceptable test results, which may indicate that the product batch in question deviates from the acceptable norm. Such occurrence is subject to investigation and a review as to the acceptability of the batch.

It is also a QC responsibility to ensure the appropriate monitoring of a vaccine for stability. The stability is generally tested at various storage temperatures from the proper freezer or refrigerator environment up to the extreme conditions to which a product can be subjected when delayed in transit. The measurements taken at the higher temperatures can also be used to predict the degradation slope at the lower temperatures using the Arrhenius Plot. Such predictions are often used for the initial determination of a reasonable dating period for the product, but it is clearly the manufacturer's responsibility to demonstrate, on a regular basis, that the vaccine indeed meets these predictions consistently when the measurements are made at the claimed temperatures and time intervals.

Distribution

The manufacturer's responsibility does not end when the final, filled, packaged and released product leaves the premises. It is mandatory to ensure that the vaccine is manufactured and packaged to withstand the handling and storage to which it will be subjected *en route* and the manufacturer must, as far as possible, be in control of the route and method of shipment.

The manufacturer must also maintain records of both primary and, if possible, secondary destinations of the vaccine to be able to institute a rapid and effective recall should it be required. (NB: When intergovernmental organizations or national ministries of health distribute the product, the manufacturer can rarely go further than informing these agencies, who must then follow up with the recall down the distribution chain.)

It is, obviously, also incumbent upon the manufacturer to ensure that all labelling and instructions accompanying the shipments are clear and concise with respect to indications, application, administration, precautions and warnings, storage requirements and any other information important to the distributor, health care personnel and user.

In practice, the ultimate responsibility for all these items rests with QA and QC and, in North America, with the Responsible Head, who is the individual responsible for all regulatory requirements and liaison related to the products.

Summary

While this chapter is not intended as a treatise on 'How to license a vaccine', it is an attempt to clarify some of the steps needed to achieve this objective.

It has only been possible, in the available space, to outline some of the most important requirements, and in doing this generalize them to the extent that the broadest possible scope of regulations are covered. Biotechnology is a highly dynamic science with innovations being introduced at a rate which is difficult to follow, let alone fully absorb. The future detailed requirements for products prepared using this technology are therefore likely to be developed simultaneously with the products themselves.

Many national regulatory bodies are staffed with highly competent scientists who are working long hours trying to keep up with scientific progress, doing research of their own and at the same time trying to assess the impact of innovations on the safety and efficacy of the resulting therapeutic and prophylactic products. Many of the regulations and minimum requirements promulgated today may be obsolete tomorrow, and some of the tests which are still mandatory make little sense in terms of today's knowledge. However, effecting a change in requirements is an arduous process, where science and logic often must yield to legalistic technicalities.

Consumers everywhere, quite rightly, want to be able to take advantage of medical developments on the frontier of science – however, only a very few will, understandably, accept any probability of risk, however statistically insignificant it may be. The developers, manufacturers and regulators of biologicals as well as those dealing with pharmaceutical products and medical devices must function within these constraints, and it is essential that these groups work together as a team. This does not imply any unsavoury concepts such as influence peddling or conflict of interest, but is merely an extension of already ongoing international and national collaboration on specific scientific, technical and regulatory issues.

Some manufacturers approach the regulators with suspicion and hostility. The meetings become confrontational and hence non-productive. It is only in an atmosphere of mutual trust and respect that the decisions and agreements, so essential to progress, can be made. It is therefore important to seek early and frequent contact with the regulatory authorities from whom licence is sought. Only by establishing, as early as possible in the development process, an understanding with the scientists and bureaucrats who are charged with the responsibility of final licence approval, can the development proceed with the assurance that at least the major avoidable pitfalls have been identified.

Equally important are the many excellent international meetings initiated and sponsored by WHO and the International Association for Biological Standardization (IABS), and by special interest groups, who from time to time promote meetings and symposia. Representatives of industry, academia, consumer groups, as well as regulatory experts should all attend and contribute to these meetings.

A project such as the antifertility vaccine is too important to be left to find its rightful place on the priority list of a single commercial company. Furthermore, it is too complex and costly to be undertaken in isolation. It is only through the frank and open collaboration between science, industry and governments that this and other major international health care projects can be completed most effectively and in the shortest possible time.

References

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13 Legal and ethical aspects of development and use of fertility-regulating vaccines

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Introduction

This chapter presents an overview of key legal and ethical aspects of development and use of fertility-regulating vaccines.[1] 'Regulation' is taken to mean contraceptive and post-fertilization limitation of fertility; fertility enhancement will not be addressed. Because of the balance of the literature relied upon for the preparation of this chapter, there will be an emphasis on issues that have been raised in developed countries, although it is realized that research is being pursued in a variety of different countries where distinctive legal and ethical issues are presented. The general level of approach applied in this chapter, particularly in its legal part, will have to be adjusted to the circumstances of individual countries.

Legal issues

Research

Background preparation – animal studies

The conditions under which primates and lower animals may be acquired or bred for research, under which they may be engaged in research and under which they may be sacrificed for diagnostic and related research are subject to legal control. Laws promoting animal welfare and prohibiting the infliction of pain on and cruelty to animals must be observed because of their inherent virtues and because observance is necessary for the maintenance of a research institution's public reputation. Public sentiment is often proportionate to the research animal's proximity to humankind. Accordingly, as research develops to involve primates, it becomes more sensitive and more highly regulated in many countries.

Laws governing the use of animals may be found in criminal legislation, and also in administrative provisions, for instance on the establishment and operation of laboratories. The latter provisions may be found in detailed subordinate regulations, and be subject to fine-tuned adjustments, perhaps made with little advance notice.

Selection of animal models may be timely only on completion of in-vitro studies, and after consideration has been given to alternatives to animals, such as computer models. Selection of appropriate animal models is legally

important to questions of legal liability for product safety and development; vaccine manufacturers may be legally accountable for selecting one course of product development and for failing to consider or rejecting the use of alternatives. Further, under traditional negligence law, the standard of care owed to anticipated ultimate users of a vaccine may be influenced by the range of models among which research might be conducted.

Contraception, contragestion and abortion research

In most legal systems, significant distinctions exist between contraception and abortion.[2] Contraception is frequently regulated on grounds of safety and effectiveness, and to a lesser extent on access to services, while abortion tends to be regulated on moral grounds. Abortion regulation often centres more on legitimacy *per se* than on effectiveness and patient welfare, including counselling. Research intended to achieve contraception is therefore likely to be subject to regulation by scientific criteria, while research on abortion, particulary by medical means, is likely to be regulated as well and even instead by laws on criminal liability and professional misconduct.

The recently perceived practice of contragestion, meaning use of post-ovulatory methods[3] designed to prevent the initiation or the completion of implantation of the pre-embryo in the uterus or to induce the early failure of gestation of the implanted embryo, commonly clouds the distinction between contraception and abortion. The law applicable to contragestion varies in clarity and specificity in different countries,[4] but for the greater protection of research institutions and personnel they may choose to consider themselves bound by the abortion law of their jurisdiction. If the result is found too restrictive of research, appropriate officers may seek prior informal approval of contragestive protocols from legal departments or officials of government, for example an Attorney General, a director of legal services of the Ministry of Health, or a senior prosecutor. If a reply is not forthcoming, the prior disclosure of intent to undertake such research will be legally advantageous.

The legal duty of care owed to research subjects requires appropriate regard to be paid to their alternative means of access to contraceptive, contragestive, or abortifacient services. Potential subjects' options of access to services have to be assessed before populations can be targeted for invitation to participate in research studies. The selection of independent as opposed to dependent populations is a matter more of ethical than of legal control of research, but evidence that a conveniently accessible population was selected without consideration of a scientifically equally valid alternative population for recruitment of subjects may reinforce an injured subject's later complaint of legal negligence in study design and execution. In particular, care must be exercised not unduly to recruit less educated, well served or age-appropriate subjects.

Measurements of success of vaccine research must identify research failure as consisting of either unwanted pregnancy or unwanted infertility. That is, restoration of fertility is as much a measure of success as prevention of

unwanted or continued pregnancy. Contraception, contragestion and abortion must be distinguished from sterilization, and the risks of both unwanted pregnancy and later inability to conceive and gestate a wanted child must be addressed in a study design.

Clinical trials

Research is designed to achieve generalizable knowledge and the outcome of treating an individual research subject is significant primarily for its contribution to this end.[5] In contrast, therapy is designed to assist the individual patient, although its results may be of secondary interest to the research community. Where research is undertaken on patients seeking care, the functions of research and therapy may coincide, but they remain distinguishable. Treatment intended for a patient's benefit may produce research data, for instance of anecdotal interest or to contribute to retrospective study of therapeutic outcomes. Prospectively designed studies of therapeutic outcomes must be approached primarily as research. Confusion must be avoided between a physician's role as a researcher and as a therapist primarily committed to a patient's welfare. Equally, a patient must be distinguished from a subject of research, although a subject may also be a patient, particularly when the investigator is also the subject's physician. Where the primary or predominant purpose of the interaction between the investigator/physician and the subject/patient is acquisition of data for research, notably when proposed management complies with and is intended to serve a prospective research design, the transaction must be considered to constitute research, and the parties must consider themselves to be respectively investigator and subject. Any claims and privileges arising from their status as therapist and patient will accordingly be of no legal significance. Indeed, it would be negligence in law for an investigator to permit a subject to believe that a purely therapeutic goal is intended when the subject is treated, or is proposed to be treated, within a prospective research design.

Therapeutic innovation arises when prevailing therapies are discredited or contraindicated, but investigation occurs when research is undertaken in a new therapy intended to advance the quality of care.[6] Use in a human subject of, for instance, a contraceptive vaccine, may be therapeutically intended for the subject, but will be primarily designed to produce data of value to a research project. In view of contraceptive alternatives likely to be available to the subject, a legal duty exists to make clear to the subject that he or she is proposed to be involved in research, and any privilege that a therapist is legally empowered to exercise to withhold countertherapeutic information from a patient will be inapplicable in seeking the subject's consent.

Informed consent

An important principle of law is that nothing be directly done to individuals' bodies without their prior consent.[7] Failure to observe this principle can result in legal liability for criminal assault and compensible battery. The principle

serves the ethical goal of autonomy, and confirms that the decision whether or not to have medical treatment is not in itself a medical decision, however much the treatment may seem to be medically indicated. The decision is a personal one, which the law requires to be informed through the offering of appropriate medical and non-medical information.[8] Particularly important to some people is their different response to contraception and to abortion, so that treatment that has or that may have an abortifacient effect must be clearly identified to them as having that potential.

Legal principles of informing for medical choice are specific to each jurisdiction. In general, a distinction exists between legal systems that require a health professional to observe the standard of conduct that would be observed by peers (that is, the so-called 'professional standard' of disclosure), and that require information to address the issues that a reasonable person in the patient's circumstances would consider material to know (that is, the so-called 'objective patient standard').[9]

Whichever orientation or standard of disclosure is legally required to be observed, information should normally be supplied to prospective subjects of research that includes:

(i) the fact that they are being invited to participate in research;
(ii) their therapeutic options, where they are eligible to receive therapeutic care;
(iii) the consequences of them not participating in the research proposed (for example, the results of them remaining untreated and their preservation of options of therapy);
(iv) the known effectiveness of their research and therapeutic options;
(v) the known risks and side-effects of their research and therapeutic options; and
(vi) the extent or nature of material information regarding prospective research and therapeutic options that is unavailable.

If clinical judgment about a patient's treatment is being suspended in a proposal to engage the patient as a research subject, for instance in an invitation to participate in randomly determined treatment, the patient/subject shall be so informed. Similarly, if for the duration or a period of the research the subject will receive or may receive treatment not intended to be medically active (that is, placebo treatment), the subject shall be so informed. If the subject's individual treatment will be of a type of which the agent who applies it and/or the person who measures or assesses its effect is unaware, the subject need not necessarily be informed in law, provided that the code allocating the subject to a particular treatment can be broken and the subject identified upon a sufficient indication of harmful or non-protective effect. If such an indication is not predetermined and/or closely monitored and the subject may be exposed to any risk, the information about breaking anonymity shall be included in the information of risk to be given under (v) and (vi) above.

Reproductive research bears a particular legal burden because it involves subjects who are anticipated to be sexually active and who intend to conceive children at a time of their own choice. Such subjects may legally agree to accept the risks of their own involvement in research, but they cannot consent to terminate or limit legal claims that will be available to affected children they subsequently bear. In some jurisdictions, moreover, particularly in the USA, legal liability is owed to at least viable fetuses that suffer stillbirth due to negligence.[10] A subject of research cannot give an investigator consent that will protect the investigator against a claim brought by or on behalf of a born child or a viable fetus alleged to have been injured due to the investigator's wilful or negligent breach of duty of care.

Free consent

In addition to the legal requirement that consent to participation in research be adequately informed, is the requirement that it be freely given. Both requirements must be observed in order to satisfy the underlying ethical value of autonomous choice. Freedom of choice is clearly violated when coercion or pressure is brought to bear on a subject, by the investigator, another member or supporter of the research team or, for instance. a spouse or parent. A more subtle violation of freedom occurs, however, when a prospective subject is induced to collaborate in research. The distinction that exists in theory between permissible and excessive inducement is difficult to apply in practice, particularly regarding populations deprived of routine services for regulating their fertility. Such populations may be identified by reference to their socio-economic status or, for instance, their place of residence, but they may also be found as subgroups of populations whose members enjoy legal independence of choice, such as young people and less-educated people.

A particular problem regarding routinely deprived or under-serviced potential subjects is that they may respond to the offer of medical attention by compliance with whatever is asked of them. Their desperation to receive medical attention and treatment for themselves or their children and families may make them anxious to establish links to health professionals that would be forged by participation in research. The problem is particularly acute concerning disadvantaged women with young children and limited access to health services because of poverty or, for instance, geography. Research institutions may be unable to liberate consent from such inducement by offering therapeutic care for families of potential subjects, but still bear the legal burden of showing that any inducement was inadvertent and that the research did not involve an unreasonable risk of injury or a risk that fully free persons invited to participate in the research had refused or failed to accept.

Subjects who are patients may lack adequate freedom of choice when invited to participate in research in which principal or collaborating investigators are their physicians. Consent is particularly suspect when given at the request or invitation of such physicians, but even in other cases a subject's agreement to assist an investigator on whom the subject is dependent

for health care is legally of doubtful effect unless the physician/investigator can show that the subject took, or at least was aware of means of access to, independent advice. A common principle of law in unequal relationships such as between physician and patient and lawyer and client is that when an advantage flows from the weaker party, the patient or client, to the more powerful party, the physician or lawyer, courts will consider that there has been an exercise of undue influence, and will treat the agreement as voidable unless the more powerful party can prove that no undue influence was applied deliberately or inadvertently. Where a patient is dependent, or feels indebtedness or gratitude, including spontaneous gratitude for care, a physician may be unable to show the patient's adequately free consent to act as a subject. Although a patient's trust in a physician will not in principle invalidate consent, courts are aware that patients may not perceive the transition from therapy to research or their physicians' functions as investigators. Unless such investigators can show that their patients who were recruited as subjects were adequately informed of the different functions and interests arising from the research, and were able to keep their therapeutic associations without fear of loss or prejudice if they declined or withdrew from participation in the research, courts may find that the patients' consent to be subjects was not freely given.

Adolescent consent

Capacity to give informed and free consent to research may be compromised by youth.[11] Many legal systems appear to deny a legal capacity to consent to those beneath the age of majority or a lower age applicable to medical treatment. Courts may consider such age to bar independent consent, but a growing tendency is to consider such age to raise only a presumption of incapacity that can be rebutted by evidence of individual capacity in prevailing circumstances.[12] Courts may then find that particular adolescents under age are able to exercise autonomy on regulation of fertility. The pressure resisting this tendency in the law is the parental and social will to control not simply adolescent pregnancy but adolescent sexual activity.

Adolescents not capable in law or fact of exercising autonomy may fall under control of their adult guardians, usually their parents. Guardians thus may veto the participation of adolescents in research. When guardians volunteer adolescents for research, however, the danger of such subjects' lack of free consent obviously arises. Where guardians' consent is in law a necessary condition of adolescents' participation, it is not in itself usually a sufficient condition.[13] Many jurisdictions recognize the legal significance of the adolescents' own assent, by which they show their willingness to accept any discomfort and inconveniences that the research may entail for them. If, on being informed of what they must endure for the sake of the research, they decline participation or refuse collaboration, for instance by refusing to accept injection of vaccine, they have not assented and cannot legally be subjected to treatment, notwithstanding their guardians' approval.

Gamete acquisition

When research requires control or manipulation of sperm or ova *in vivo*, consent is legally required in accordance with the principles outlined above. When gametes are required, however, for in-vitro study, their acquisition is regulated by different though related principles.[14] A number of jurisdictions have legislation governing donations of human tissues for research, which must clearly be obeyed, although many jurisdictions' laws apply only to post-mortem donations of tissues, or sometimes only of solid organs. In the absence of controlling legislation, legal principles will usually require that gametes be acquired with free and adequately informed consent, as discussed above.

Women must be informed and freely consent, for instance, if it is proposed that they receive medical treatment to stimulate ovulation or superovulation for recovery of ova for research. More questionable is whether such consent is necessary for recovery of any ova found in pathological waste resulting from hysterectomy, although consent will be required if the donor's anonymity is not fully ensured. Sperm donors should in principle be treated equally, although consent may adequately be given when a sperm donor contributes a specimen to an infertility clinic, because he may be found to have abandoned the sample to the clinic subject to anonymity in its use.[15] A man who gives a sample as a patient does not necessarily consent to research use, however, and a separate consent should be sought. The same is true regarding an ovum-donating patient, and both genetic donors of a pre-embryo available for research, for instance as a result of their participation in in-vitro fertilization programmes.

Liability for injury

Vaccines must be tested *in vitro* and in animals before being tested on human subjects. Any negligence in the conduct of such testing that causes injury, or that prevents awareness of risk of injury to human subjects who are hurt, may be legally actionable. Since one purpose of human testing is to determine the risk of failure of the vaccine, for instance through unwanted pregnancy or sterility, and the risk of injury or discomfort as a side-effect of use of the vaccine, subjects of testing must be informed to a high degree of their liability to suffer injury. If they knowingly and freely assume that risk, they may have no legal cause of action (but see also 'Compensation and insurance' section, below). Although injury may occur when there was no legally actionable negligence in their management, a number of jurisdictions may find negligence if subjects were inadequately informed of risk and were thereby caused to consent to treatment that they would otherwise have declined.

Negligent injury from use of an approved and marketed product is often traceable to negligent design and conduct of research studies. Such negligence may appear not in the aftermath of treatment of an individual research subject, but in the course of long-term monitoring of such a subject, or of the subject's children. Legal standards are set for the conduct of research through cases concerning not only research subjects themselves, but also ultimate

consumers and the children of both subjects and consumers. It has been seen above that adults who are research subjects, as is true regarding consumers too, cannot legally surrender the rights of legal action that their children may come to acquire. Care must therefore be exercised in research to monitor related defects in the offspring, stillbirths, abortuses and products of conception of animal and human subjects.

Unwanted pregnancy and birth

It is a matter as much of aesthetics as of law whether courts will regard conception of a child as a legal injury. Conservative courts tend to regard childbirth as a blessing that transcends and overcompensates any legal negligence, and will either dismiss a negligence action for unwanted pregnancy and birth or decline to award damages. Increasingly, however, courts are willing to accept that negligence resulting in unwanted or untimely pregnancy is legally actionable[16] whether pregnancy results in childbirth, stillbirth or induced or spontaneous abortion. The particular outcome of pregnancy will affect the calculation of damages awarded, but not the underlying principle of legal liability. Pregnancy cannot be considered an unanticipated or separately arising result in research subjects selected because of their fertility and sexual activity.

Some courts may give a token award but otherwise refuse to compensate birth of a healthy child, but will award parents the extraordinary costs of maintaining a seriously handicapped child. Courts may limit such damages to the time when parents have legal responsibility for maintenance of their children. Courts differ on whether handicapped children may recover damages in their own right. Some distinguish avoidable from unavoidable injury, and will compensate the former. They will not compensate the latter, holding that, while courts can measure the difference between normality and abnormality they cannot measure the difference between abnormality and 'the utter void of non-existence'. They will therefore refuse to compensate the handicapped child the only alternatives to whose birth were that the child would not have been conceived or would have been aborted. Very few courts have accepted claims by such children, sometimes described as 'wrongful life' actions.[17]

Compensation and insurance

Governing principles of compensation and insurance for vaccine products will be addressed below in the context of the marketing and use of such vaccines.

In the setting of research, some countries' laws or guidelines require that, as part of the legal obligation to inform potential subjects about research, they be informed whether any injury suffered in research will be treated without cost to themselves or be otherwise compensated or insured.

Application for marketing approval

Vaccine licensing authorities operate under legislation and regulations that will govern research designs. That is, the type of information that such

authorities require governs the information that research will be designed to achieve. Some countries have developed legislation and regulations containing specific provisions on how testing is to be conducted, including what procedures must be applied for scientific and ethical review and monitoring of research protocols. At the other end of the spectrum are national laws that provide little more than that before products can be imported and prescribed they shall have been approved in their countries of origin (see 'Marketing and use – Product advertizing', below).

Contraceptive products are normally distinguishable from therapeutic products in that the latter are used episodically by sick people whereas the former are used preventatively for long periods of time by generally healthy people who want to avoid the natural effects of normal behaviour. Accordingly, the testing requirements for contraceptive products are normally more demanding than for therapeutic products. They must be tested over a lengthy period of time, and their dysfunctions, meaning their rates of failure, their risks of causing injury, and their side-effects of discomfort and inconvenience, are not assessed against the alternative of persisting illness. Because they are intended for use by fertile, sexually active people who are otherwise likely to conceive children, their liability to cause teratogenic effects, genetic mutation or other congenital injury must be carefully determined, preferably over several generations of test animals. Further, such products cannot be offered to potential human users with the health professional's so-called "therapeutic privilege' of non-disclosure of any countertherapeutic or deterring information.

National patterns of product approval vary. Some laws require extensive and therefore costly pre-marketing approval before a product may be prescribed for human use. The purpose is to reduce risk to the feasible minimum, even though the effect is to deny reasonably safe and effective products to users who would benefit from them and who have few if any practical alternatives available to them. In contrast, other laws are willing to approve human use of products that have been adequately though less comprehensively tested before routine marketing, but require an extensive period of post-marketing monitoring of effects, through collection of scientifically valid and anecdotal data, and with the prospect of issue of an order that the product be withdrawn or be prescribed only to more narrowly selected populations. The result is that sound products are marketed more quickly but that a narrow margin of patients for whom the product proves dysfunctional will be identified only through the products' adverse effects.

Marketing and use

Contraception, contragestion and abortion

It has been seen above that the legal distinction between contraception and abortion, based on the imposition of abortion liability for interference with gestation after completion of the process of implantation of the pre-embryo in

the lining of the uterus, is challenged by the concept of contragestion. Abortion is often severely punishable and therefore requires a strict definition and an onerous burden of legal proof of guilt. In contrast, contraception tends to be considered a regulatory matter of medical choice.

In many legal definitions, the punishable act of abortion consists not simply of dislodging an implanted embryo but of taking action intended to dislodge any embryo that may have become implanted. Legislative systems may be divided into those that penalize treating a woman who is 'with child' and those that punish an act intended to cause a woman's abortion 'whether or not she is pregnant'.[18] A product of research on fertility-regulating vaccines will be determination of their *modus operandi*. Medically, it may be of no consequence whether they operate before or after completion of implantation, but the distinction may be of legal significance. Medical uncertainty may obstruct their employment, because if there is speculation that they may operate on the abortion side of the contraception/abortion distinction, physicians who use them may be found to be willing to terminate a pregnancy if it exists, and therefore to be punishable. A prosecuting authority can invoke medical uncertainty to deny a physician's claim of honest belief that a vaccine operates only as a contraceptive.

Some jurisdictions, through their criminal or constitutional laws, claim to respect human life 'from conception'.[19] Such laws may serve a symbolic rather than a pragmatic function and may not obstruct abortion indicated on medical grounds. That is, the claims to life and health of a woman are no less protected than are those of an embryo, and a greater social interest may be served by permitting her reproductive health choice, particularly at the early stages of pregnancy, than by subordinating her welfare to that of the embryo she bears. Laws affording recognition of human life from conception may raise difficulty in application because of problems of proof of conception, and may be challenged on the legal ground of being void for vagueness. That is, non-specific terms may be held legally inoperative. 'Conception' is determined as a matter of law as opposed to medicine, and may or may not coincide with fertilization; it would be legally feasible, for instance, to deem every woman of reproductive age to have conceived who is beyond 35 days from her last menstrual period. Unless every fertile woman who has recently engaged in sexual intercourse, and who is therefore potentially pregnant, is to fall under legal regulation and forensic scrutiny, some tests of 'conception' must be applied that are not physically invasive or emotionally threatening. The effect of a strict application of such a provision may remain, however, that deliberate development and marketing of products operative after conception to terminate gestation will be subject to laws on abortion.

Once a product is approved for marketing on prescription, physicians usually enjoy the power to prescribe it not only for the indications for which it has been tested and approved, but also for other purposes. Manufacturers of the product cannot advertize it for purposes for which it has not been tested and approved, and in principle bear no liability for inappropriate or non-

indicated use. As against this, many systems of product liability law require manufacturers to anticipate and bear responsibility for misapplication of their product that causes harm. A distinction may be drawn, however, between inadvertent and wilful misuse, manufacturers' liability applying only to the former case (see 'Injury and causation', below).

Product advertizing and distribution

Legal systems tend to govern the advertizing and distribution of pharmaceutical products and vaccines by detailed provisions with which marketing personnel are familiar. More general legal issues regarding products available on prescription concern the relationship between the prescribing physician, the patient, the manufacturer and the supplying pharmacist.[20] Vaccines are legally less problematic than drugs that are compounded by the pharmacist and are therefore liable to compounding error, because vaccines tend to leave the manufacturer in a state fit for use and are simply allocated by the intermediate supplier to the ultimate consumer subject to conditions of storage for maintenance of fitness for use. Prescription products are frequently prohibited from being advertized to the public at large, but may be advertized only to such professionals as physicians and pharmacists. Accordingly, both the indications and the contraindications for use will be advertized to these professionals. The result may be that manufacturers bear liability to ultimate consumers for use of products in accordance with manufacturers' advertizements of conditions for use, for instance when the product has a defective design, but are not liable when the product is prescribed for conditions for which it is not indicated or is contraindicated. The 'learned intermediary rule' protects a manufacturer from liability for improper use resulting in harm when the product is available only through a learned intermediary who is given information about appropriate and inappropriate application of the product and is responsible for protection of potential consumers against inappropriate use.[21]

Interesting legal questions concern the interaction of the prescribing physician and the supplying pharmacist, because physicians exercise clinical judgment and have ultimate responsibility regarding their patients, but pharmacists also have a protective role concerning patients. Pharmacists may have a duty to question an apparently inappropriate prescription, for instance where the dose seems wrong because of a misplaced decimal point or the prescription seems ill-advised in light of the pharmacist's knowledge of the patient's history of use of prescription products. If a questioned physician confirms the prescription, however, the pharmacist is entitled and perhaps obliged to complete it. The pharmacist should be wary to give advice that may counter or vary the use of the product that the responsible physician intends, or to supply information to a patient that the physician may have decided not to disclose in an exercise of clinical judgment; that is, an exercise of the physician's therapeutic privilege of non-disclosure.

Product packaging

Regarding products sold without prescription, the manufacturer communicates material information to a prospective consumer through the product package. Information outside the package may be repeated or amplified by a package insert, but communication by insert alone is objectionable and perhaps counterproductive, because a potential consumer may have to purchase and break the seal of the product before reaching the insert to discover whether or not the product is suitable. Much of the law relevant to packaging will be inapplicable to vaccines, which are usually available only on prescription and which are administered by a health professional rather than self-administered by the consumer.

A development in the law of supply of contraceptive means in some jurisdictions is that, notwithstanding the learned intermediary rule, package inserts must repeat warnings and contraindications to potential consumers before they take the product. Again, the applicability of such doctrines to fertility-control vaccines is questionable where such vaccines are not self-administered but are given by health professionals who are required to make appropriate disclosures and to determine any physiological contraindications for use in the course of conversations with prospective consumers. A requirement of written disclosure is unrealistic with illiterate populations, populations with poor reading and comprehension skills and those who are literate only in alien languages.

Consumers' informed consent

Attention given above to informed consent in the context of research presents simply a special case of the general legal doctrine of informed consent. The historic significance of consent in medical treatment is to neutralize what otherwise would be the wrong of battery constituted by unauthorized touching. Consent neutralizes battery, but in order to distinguish comprehending agreement from mere passive acquiescence, courts have tended to require that the consent have an adequately informed quality and have therefore fashioned the so-called doctrine of informed consent. Although analytically informed consent is different from free consent, and in the research setting the distinction is helpful to maintain, some analysts observe in the context of therapy that information serves the goal of individual freedom of choice, and therefore conclude that freedom of choice is a necessary element of informed consent.

In recent times, courts have approached the duty of disclosure of information material to choice as discharging duties not only under the law of battery but also under the law of negligence. The duty of care concerns not just proper informing, but also concerns the proper conduct of appropriate research to acquire material information to disclose. Accordingly, the legal principle of consumers' informed choice controls the care with which

manufacturers are obliged to undertake preliminary research through in-vitro and animal studies and research with human subjects. For instance, prospective consumers will consider material to their choice of product use its safety regarding their future fertility, risk of defects caused to subsequently conceived children or those conceived due to product failure, and the incidence of such failure. Manufacturers' misplaced assurances on these matters due to inadequate research, including inadequate long-term follow-up studies, will deny consumers informed choice and be legally actionable negligence.

Adolescent consent

The issues addressed above regarding adolescent consent to research are applicable in principle to therapeutic use of products. A distinction exists in that an adolescent's access to a vaccine of established efficacy and safety may be considered of therapeutic value to the adolescent in a way that recruitment in research is not. Alternative contraceptive or abortifacient techniques may be compromised through the visibility of the means of achieving them. Contraceptive pills may be difficult to store in privacy in a home that has relatively few objects. Periodic vaccination can serve a function of protective autonomy that satisfies the goal of health as broadly construed by the World Health Organization.

Any reduced capacity for comprehension that may affect adolescents in different cultures cannot be a legal basis of reduced or oversimplified disclosure of information. A prospective consumer who, on grounds of age, is incapable of understanding the significance of the choice that is to be made is likely to be held in law to lack capacity to make that choice, and is therefore ineligible to be the decision maker. If, however, such an adolescent is at risk of dangers including short-term repeat pregnancy, a protective initiative may be taken on the authority of an appropriate guardian. Giving such authorization is an act of paternalism that is increasingly held to be legally objectionable in several jurisdictions because of its violation of individual physical integrity and dignity. The act may be excusable where it is of short-term effect (that is, reversible) and offers a worthwhile degree of protection against the hazards of pregnancy an adolescent faces through vulnerability to social pressures to be sexually active.

Injury and causation

The definition of legal injury and principles of causation are major elements in almost every system of legal compensation. Further, in most developed systems of law, provisions on product liability form a major and discrete body of jurisprudence, sometimes reflecting internationally comparative law.[22] Within the European Communites there is momentum to achieve harmonization of product liability law in member states.[23] Much of this law is instrumental in that it is concerned with the promotion of good outcomes and

the suppression or allocation of risks. At the same time, however, both judicially declared laws and legislated provisions can be ideological in that they rest upon ideologies of causation of injury and incentives to the exercise of care. They also reflect an assessment of the value of a product in that disfavoured products can trigger an extent of liability that discourages their supply while favoured products are protected in order to encourage supply. For example, in the USA, product liability actions have almost eliminated the supply of intra-uterine devices (IUDs) while the desirability of a supply of transfusable blood has persuaded courts to protect blood supplies from product liability suits.[24]

Fertility-regulating vaccines may be considered comparable to IUDs in that courts may feel no special incentive to give protection against the deterrent effect of rigorous application of product liability laws. Contradictory reasoning may be based on the claim that contraceptive alternatives are decreasingly available (such as the IUD itself) or are more hazardous in long-term use, and that, for populations in general or for more targeted populations, such vaccines are preferable to pregnancy. Abortifacient vaccination is liable to be assessed in the context either of the safety and efficacy of alternative abortifacient means, or of the desirability of abortion itself. As against all of these considerations, however, fertility-regulating vaccines may be analogized to transfusable blood, and receive generous legal protection as constituting a benefit for eligible consumers.

It has been seen that courts take different approaches to whether the birth of a child may be accepted as a species of legal injury, courts sometimes distinguishing between births of healthy and of handicapped children. Negligence may be found to have caused childbirth where favourable information about a contraceptive vaccine induced a consumer to forego alternative contraceptive means or to abandon sexual abstinence and risk pregnancy. In the special area of vaccine-related injury, however, causation of injury can be claimed to have occurred in several additional ways.

Vaccine related injuries

The pattern of litigation shows that adverse physical reactions to vaccination (or 'immunization') have led to litigation based on different claims of liability. Errors in the manufacturing process may be alleged, such as contamination, adulteration or improper configuration. Inadequate testing may be alleged, such as pursuit of an inadequate research protocol or negligent (or even deceptive) execution of an adequate protocol. In addition, improper labelling or informing of physicians, pharmacists or others may be alleged. If a vaccine is not claimed to have been defective in itself, injury may be alleged because the vaccine was administered improperly or in an improper dosage, due either to operator error or to the manufacturer's failure to instruct operators appropriately. Similarly, it may be claimed that application of the vaccine was contraindicated either in general or in the circumstances of an individual, and

that the fault is that either of the operator or of the manufacturer in failing to provide the operator with appropriate instructions (perhaps because adequate protective research was not conducted or was negligently conducted). Contraindications may consist of consumers' allergies, illnesses, immunosuppression or age.

It is legally recognized that non-defective vaccines that are properly administered can result in unforeseeable adverse reactions, and laws must determine as a matter of policy what approach they will take to claims by injured consumers. Classically, a plaintiff bears the legal burden of proving the defendant's fault, and non-fault-based injuries will be uncompensible. Under modern jurisprudence on product liability, however, courts may impose so-called strict (or 'no-fault') liability where products are found to be unreasonably dangerous.[25] Some products are found to be unavoidably unsafe, in that they present irreducible minimum risks of causing harm, but not unreasonably dangerous. Accordingly, courts may impose strict liability on manufacturers of unreasonably dangerous products, but may find that vaccines that are properly tested and marketed are only unavoidably unsafe. Plaintiffs will therefore bear the ordinary burden of establishing express negligence as having caused their injuries if they are to recover compensation. When a product is found unreasonably dangerous, however, the manufacturer bears legal liability for the injuries it causes, but may be able to show some contributory (or 'comparative') negligence by the plaintiff such as disregard of instructions, that reduces liability to pay compensation.

Compensation and insurance

Injury may be shown to be related to vaccine use under principles of fault-based liability, either because the vaccine caused the injury or because defective information caused the victim to accept the vaccine and suffer its irreducible minimum risks when proper information would have resulted in the victim not accepting the vaccine or the vaccine would have been used in a different way. Under principles of strict liability, the victim may claim to have suffered injury following use of a product legally determined to be unreasonably dangerous. In either case the victim will ask for financial compensation.

Whether liability is established on the basis of fault or strict liability, courts will determine the injured consumer's entitlement to compensation.[26] Courts will be sympathetic to the pain and suffering of physical injury, and will include in awards itemized claims for medical costs, lost wages or earning opportunities and, for instance, costs incurred in providing care for existing children and for maintaining the home when help was not made available without charge. Itemized expenditures for goods and services related to the injury tend to be compensated as a matter of course, but courts take different approaches to compensation of pain and suffering. Some decline compensation or award it at very modest levels because it is vague and easily simulated,

and others accommodate compensation in principle but subject it to a ceiling designed to meet reasonable claims but not to amount to a lavish reward. Yet others resist judicially set or legislated ceilings, however, and place no limit on awards that may be made by judges or juries. Beyond compensation, some systems permit the award of punitive or exemplary damages, designed to demonstrate society's condemnation of the defendant's callousness, gross negligence or, for instance, greed for profits.

More problematic is compensation for emotional harm. In a sense, pain and suffering are a species of emotional harm that courts have traditionally compensated, but usually as a secondary element of claims for physical injury. Where no physical injury is shown to have been caused, or to be compensated under principles of strict liability, injured feelings, lost opportunities for future satisfaction and other forms of emotional or so-called 'psychic' injury may not be independently compensible. Several jurisdictions are abandoning historical inhibitions based on the risk of simulated or otherwise abusive grievances, and are recognizing a legal right to peace of mind and to protection against suffering, upset and humiliation.[27] If an injury with no physical aspect can be shown to have caused clinical depression or other psychiatrically diagnosable condition, the claim will probably be more credible where psychiatric expert witnesses are customarily respected in the courts, but undiagnosable injuries with which courts find that they can identify themselves, such as lost self-esteem and lowered morale and expectations of future happiness, and feelings of guilt and remorse, may be compensated.[28] If a fertility-regulating vaccine leads to sterility or, for instance, to an inadequately informed woman perceiving that she induced her abortion, compensation for emotional injuries of this nature may be ordered against the manufacturer, prescribing physician or other party such as the physician's employing hospital or clinic.

Because of prospective manufacturers' fears, particularly of strict liability and extensive damage awards, which may induce them to decline to initiate or to discontinue manufacture of vaccines, several jurisdictions have introduced legislation to provide for state or other provision of compensation for injured consumers, and/or for limitations on recovery of compensation. This has been found regarding vaccines for neonatal conditions and childhood diseases, and has been proposed to encourage and protect development of a vaccine for the acquired immunodeficiency syndrome (AIDS) virus.[29] It is a matter of judgment whether the urgency or desirability of developing a fertility-regulating vaccine warrants this degree of protection of a marketable product.[30]

Injured consumers not wanting to be dependent for compensation on litigation or on governmental provision of funds may seek private health protection insurance. Premium levels would be actuarially set according to information the insurance industry possesses of the extent and frequency of predictable injury. The industry tends to be conservative, meaning over-protective, in its estimates of the funds it must accumulate to cover injuries that may occur. Insurance policies tend to cover only provable losses or costs,

and not cover awards for pain and suffering except perhaps at modest token amounts. In many countries, health insurance is a social service[31] and systems of social insurance cover payments during unemployment or disability and for retirement, so that private insurance has a secondary or even a legally prohibited role. More significant than consumers' means of insurance may be manufacturers' means. Where these are inadequate to induce manufacturers to undertake or continue vaccine supply, governments may have incentives to provide funds, or alternatively to become vaccine manufacturers. Again, however, this depends on the social significance that is given to development and availability of fertility-regulating vaccines.

International implications

Vaccine import and export

While laws in countries that have developed pharmaceutical and vaccine-producing industries tend to contain specific provisions for the conduct of testing, laws in countries that have conventionally been importers of such products tend to lack detail. They may indeed provide little more than that a product is not importable unless it has been approved in its country of origin. The effect of this is that the potential importer acquires for itself the benefits of the sophisticated screening system for safety and efficacy of the product that is applicable in the country of manufacture.

A variant of this pattern of product development and importation has appeared in recent years. A potential importing country that set a criterion of importation that the product has gained approval in the country of origin may intend this to mean approval for use by the domestic population of the manufacturing country, but 'approval' may alternatively mean that the product is approved for export by the country of manufacture even though the product is not approved for use by its own population. Conditions of such export are usually that:

(i) the product has met initial standards of national testing;
(ii) an application is in the pipeline leading to full national approval;
(iii) the product has been approved for domestic use by the country to which it is to be sent.

It may at first appear questionable that a country of manufacture should approve the export of a product not yet released for general use by its own population. The incentive to approve the export of nationally non-approved pipeline drugs that are approved by the importing country is legal respect for the importing country's national sovereignty and responsibility to make decisions for the health and welfare of its population. A limitation exists in that the policy is applicable only to 'non-approved' drugs, and not drugs that have been disapproved for use or withdrawn from use in the country of manufacture.[32]

Potential importing countries that do not want different standards of

product availability for their populations than apply to the populations of manufacturing countries can bar importation of products that the manufacturing countries do not allow their own domestic populations to use. It is occasionally the case, however, that a product that is unsuitable for one population may be suitable for another. This may be because of different national disease patterns, or because of different ethnic, genetic or cultural features of the population, or, for instance, because availability of alternative treatments and safeguards against misuse create different results of risk-to-benefit assessments in different countries. A product that is subjected to particularly rigorous pre-marketing scrutiny in the country of manufacture may appear so advantageous to another country's population, perhaps when it faces otherwise uncontainable endemic disease, that the government of that country considers that the product should be imported without the more extensive testing that the country of manufacture requires.

It may appear unconscionable that a country should decline to allow exportation of a potentially therapeutic product to a sick population when its own population is not suffering from that sickness and therefore presents no urgency or pressure to approve the product. Some countries have specific exceptions from their ordinary policies of exportation that specifically accommodate exportation of products that may assist the control of tropical diseases. Growing awareness and international responses to the AIDS pandemic may liberalize exportation provisions, and facilitate exportation of fertility-regulating vaccines to countries where population pressures are particularly acute. It is a matter of national judgment, however, whether the population consequences of AIDS will counteract tendencies towards population growth.

There is a body of principles of international law applicable to exportation of therapeutic drugs and vaccines. Respect for the sovereignty of importing countries has been mentioned, and is a key principle. Under international legal doctrine on state responsibility, a country has an international obligation not to permit the use of its territory in ways that harm other countries, and therefore must use due diligence to prevent the manufacture of products likely to cause injury in other countries. It is for a recipient country itself to determine whether or not an imported product would be injurious. Countries should have means to agree upon questions of importation and exportation of products, and to classify whether products or services are beneficial or unduly hazardous, primarily by reference to the decision-making authority of the country whose domestic population is liable to be affected.

State sovereignty is coming to be affected in some areas by the development of international minimum standards. That is, countries will be expected and perhaps eventually legally required to exercise their sovereign rights and to discharge their state responsibilities in accordance with international treaties, agreements, codes or guidelines that set minimum standards of conduct.[33] Prevailing models affect product assessment and post-registration monitoring

of products[34] and include the International Code of Marketing of Breast Milk Substitutes (1981) and the Recommendations of the International Population Conference (1984), which include a recommendation that governments take steps to help women avoid abortion. The development, exportation and importation of fertility-regulating vaccines may serve the purpose of this recommendation.

Liability and compensation issues

When products manufactured in one country are associated with injuries suffered by individuals in other countries, the question may arise of legal liability. The claim that the manufacturing country's lax standards of approval for marketing and exportation was the cause of injury to nationals of another country may be the basis of a claim in international law where the injured nationals' country takes up the claim as an injury to itself. More probable, however, is that the claim will be assessed under legal principles not of Public International Law but of Private International Law (sometimes described as Conflict of Laws). Each country has its own body of private international law doctrine, governed as other aspects of domestic law by judicial precedents and legislation. Because of the obvious international flavour of this area of domestic law, however, it is particularly liable to be affected by international agreements on how domestic legal systems should respond to particular issues. An incident of alleged harm may be legally actionable in more than one country, creating the possibility of a plaintiff engaging in 'forum shopping'; that is, selecting to litigate in a jurisdiction that seems to offer greatest advantage.

If, for instance, the courts of one country have held that the birth of a healthy child may be recognized as a species of legal injury, while the legal precedents in another country in which suit might be brought preclude such a ruling, a person claiming negligent manufacture or use of a contraceptive vaccine resulting in birth of a healthy child will clearly prefer to sue in the former country rather than the latter. Similarly, where the courts of one country in which suit might be brought will compensate a proven injury more easily or more generously than another, potential plaintiffs will be attracted to them. Comparative analyses of compensation programmes for vaccine-related injuries in different countries and in different jurisdictions within single countries are valuable to the exercise of such legal choices.

Potential litigant choices may be affected by a variety of policy factors that apply beyond the specific laws and legal principles of courts of particular jurisdictions. Where governments wish to encourage particular industries, they may afford them protections by creating legal immunities from suit, or by legislating product liability principles that favour manufacturers over classes of potential plaintiffs. Similarly, governments may discourage particular manufacturing enterprises by imposing strict principles of liability for defective products or by imposing penalties payable to injured consumers,

such as minimum lump-sum awards of double or treble damages. Some groups hostile to manufacture of contraceptive, contragestive and abortifacient products advocate legislation of punitive awards in civil actions presented by consumers who successfully allege that the products caused them injuries.

Courts themselves may decline to exercise their jurisdiction in favour of hearing plaintiffs' claims not only when they rule that they lack jurisdiction over the claims on legal grounds, but when they consider themselves not to be convenient courts or the more convenient courts for trial of the actions. For instance, a court in the USA declined to exercise its jurisdiction regarding the Indian Bhopal tragedy by invoking the discretionary rule that it was not the most convenient tribunal for the action (the *forum non conveniens* rule). If the claim centres on defective study design or testing of a vaccine, the jurisdiction where the testing data or personnel are located may present the convenient forum. If, however, the claim centres on negligent administration of a vaccine, the place where the administration occurred and where evidence of the circumstances of its administration exists may seem to offer the most convenient location for trial. Similarly, if the claim concerns special characteristics of medical treatment of an injured patient, and the information available to treating personnel, the acquisition of oral testimony may govern where the trial is best held. Documents and expert testimony can be made available to courts with relative ease in the modern world, but where evidence depends on personal testimony and the cross-examination of witnesses by properly prepared legal counsel, convenience may favour taking the legal process to where the witnesses are rather than taking the witnesses, perhaps at great cost, inconvenience and discomfort, to where a plaintiff considers it personally preferable to sue.

Ethical issues

Principles

Bioethical discourse in the English-speaking world and perhaps in western countries tends to derive its structure initially from the basic principles set out in the USA in 1979 by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research in the publication *Ethical Principles and Guidelines for the Protection of Human Subjects of Research*, better known as the Belmont Report.[35] This report summarizes and simplifies basic ethical principles that reflect western philosophies that have developed in preceding centuries, and which are applicable far beyond the field of medical and behavioural research. The three basic principles are:

(i) Respect for Persons, including:
  (a) the principle of Autonomy, and
  (b) the principle of Protection of the Vulnerable;

(ii) Beneficence;
(iii) Justice.

The principle of respect for persons, expressed primarily in respect for the autonomy of people with the intellectual capacity to understand the choices they face, has traditionally been resisted in westernized medicine, where it has been subordinated to paternalistic practices. Western medical paternalism appears comparable to that experienced in other parts of the world that suppress egoism in favour of interdependency and communal or family-centred decision making.[36] Autonomy of patients can create ethical dilemmas for physicians, because wisdom is not a precondition of autonomy. Patients are accordingly free to make informed but nevertheless poor choices, perhaps against physicians' advice, while physicians observe the principle of their profession: 'Do no harm'. Accordingly, physicians and other health professionals present information, choices, guidance and advice to patients, but decreasingly tell them what they should do, and refrain from imposing treatment that health professionals prefer except perhaps in emergency cases. The legal rules on informed consent to health treatment serve and reinforce the ethical principle of autonomy.

The second aspect of respect for persons, namely the ethical obligation to protect the vulnerable, has clear application to children and infants, but is more problematic when applied to adolescents. It may be ethically appropriate to approach adults with the presumption that they are capable of autonomy and to approach children and infants with the presumption that they are not, and to adjust behaviour according to the evidence then presented, influenced by the invasiveness of the proposed treatments, the medical circumstances and other pertinent factors. It may be ethically appropriate to approach adolescents, however, with no presumption, and to determine responses according to the degree of individual maturity, individual comprehension, urgency and risk that is found. Treatment should be guided by the principle of preserving the individual's future options and avoiding the possibilities of irreversible outcomes.

The principle of beneficence goes beyond the passive ethic of 'Do no harm' by asserting an affirmative duty to take initiatives to do good. Medical research is usually taken to serve this ethical ideal when conscientiously pursued. Beneficence requires that possible benefits of a medical intervention be maximized and that possible harms be minimized, occasionally at the cost of declining to undertake the intervention. The challenge beneficence poses to research is that the maximization of benefit may compel studies that include an element of risk of harm.

Justice is a concept invoked by the disciplines of both ethics and law. It consists in such obligations as to treat like cases alike, and appropriately to recognize contrasts between different cases. The application of justice presents the same difficulties in ethics as in law because of the need authoritatively to

classify what cases are alike and what cases are different. The law commonly enjoys an advantage over ethics in that it has tribunals and procedures to determine the decisive characteristics or properties of actions or circumstances, and to rule on comparisons and contrasts. Equal treatment of people who are equally situated, and due acknowledgement of differences among those who are differently situated, can be undertaken by a variety of approaches. Common approaches include:

(i) to each person an equal or identical share of a resource;
(ii) to each person according to individual need, as determined by an agreed, acceptable or authoritative agency;
(iii) to each person according to individual effort, determined without distinction but sensitively to individual capacity;
(iv) to each person according to societal contributions, determined as in (ii) and/or (iii) above;
(v) to each person according to merit, determined as in (ii) above.

Different levels of ethical assessment have been identified. These include:

(i) the microethical level, which deals with person-to-person relationships and has been the traditional basis of western and religious ethics;
(ii) the macroethical level, which is concerned with societal interactions, including how society regards the individual, and with the duties the individual feels towards his or her community and wider society, and the claims the individual has on such collectivities;
(iii) the mesoethical level, which has recently been perceived to exist between the microethical and macroethical levels, and to address administrative or management issues primarily of public resource allocation and institutional integrity;[37]
(iv) the megaethical level, which transcends the concerns of human communities, societies and nations, and addresses the nature of human interaction with the environment and animal and plant species and, for instance, the effects of regional and global population size and urbanization.

A modern sensitivity in the area of ethical discussion concerns whether, and if so to what extent, women possess rights and duties, individually and/or collectively, that are different from those possessed by men and by such collective enterprises as families, communities, societies and nations.[38] A contrast has been drawn between two major styles of reasoning about ethical problems that are sometimes associated with an alleged contrast between male and female reasoning, or between objective and subjective reasoning. The contrast is between:

(i) justice-based reasoning, which entails approaching and solving problems according to abstract, rationally derived rules;

(ii) care-based reasoning, entailing responses to the needs of particular persons in their circumstances.

The former is embodied in the concept 'let justice be done though the heavens may fall'. It adheres to principle, notwithstanding costs to individuals and institutions. It may be praised as principled and courageous, but may also appear callous and indifferent to individual misfortune. The latter is sensitive to circumstances including emotional attachments between persons, but may seem to break rules, be arbitrary and to compromise principles and perhaps virtue itself. Experience indicates that it is incorrect to consider that the two approaches contrast men and women, and that we all draw on both types of considerations in determining action.[39]

Human reproduction, particularly concerning the control of human fertility, is increasingly considered to be women's business. As women have emerged from the legal control of men such as husbands,[40] fathers and community leaders, the role of men in management of fertility as physicians, other health professionals and counsellors has become increasingly perceived as the application of its value system by one sex to the other. Whether or not sexual stereotyping occurs between the sexes is a matter of individual assessment, but it appears that the field of human reproduction cannot be ethically analyzed without attention to different sex roles and to interaction of the sexes.

This provides a context within which the basic principles presented in the Belmont Report are applicable. Autonomy requires that women's preferences should govern their lives no less than men are self-determining. At a macroethical level, however, women in ordinary circumstances of life may appear educationally, economically, culturally and otherwise disadvantaged and vulnerable to control or manipulation by others. Such others may be men, but also advantaged socio-economic classes. It is not obvious who might be the protectors of such women, although social histories may indicate who in the past have acted as protectors. The principle of beneficence may urge that women be spared the hazards of bearing children too early and too late in their reproductive lives, as well as too frequently and at too short an interval since the last pregnancy.[41] The principle of justice may require that women receive an equal proportion of health resources, but also that they receive care proportionate to the extra burdens that reproduction imposes on their bodies and opportunities in life.[42]

Research

Background preparation

It is now expected in research centres that there will be independent scrutiny of proposals on ethical grounds. Ethical review committees may be legally mandated and regulated, or may alternatively be required by research funding agencies, universities and, for instance, hospital accreditation agencies. Even

where guidelines for ethical conduct lack a legislated basis, compliance with their principles and procedures may be an implied term of an investigator's contract with a research facility or an implied term of a contract of employment with a university. Ethical guidelines may thereby acquire the force of private law.

Research ethics committees operate internationally under different names and structures. USA Institutional Review Boards (IRBs) are formalistic and operate according to a detailed regulated specification, with additionally demanding standards of operation the more sensitive the work they scrutinize. Less formal committees exist elsewhere to review research on ethical grounds, including general hospital committees and *ad hoc* committees acting under the aegis of, for instance, medical research councils, other government agencies and universities. At times, commercial pharmaceutical and related companies create ethical review committees composed of independent distinguished scientists and lay people, or try to obtain ethical screening from reputable university committees, such as those in whose laboratories or hospitals products are tested.

The composition and functioning of research ethics committees vary among countries and often within countries. It is important that relevant medical specialties be either represented on or be conveniently accessible to committees, and that members who are themselves investigators should have no conflicting interest, such as through competition for funds or appointments, and should be able to respect confidential scientific information, for instance regarding methodologies and results of pilot or other preliminary studies. Committees should include at least one person familiar with ethical discourse and the literature on ethics in human experimentation. Lay persons such as patients might also be represented on committees, although this raises questions about their comprehension; their special merit, however, may be that they are representative of those who may be presented with the information sheets and consent documents intended for patients, which the committee is responsible to inspect.

Research ethics committees can usually consider only protocols that are submitted to them. They do not consider the research that is ethically necessary, desirable or feasible to undertake but which is not being pursued by applicant investigators or others. The ethics of overall research planning are macroethics which might be addressed at a level of national political or other public accountability. The question will arise in scientific assessment regarding a single protocol of whether the state of existing knowledge shows the research protocol to be timely, premature or a duplicate of studies already initiated or completed. Commercially funded research may indeed duplicate studies that have already resulted in marketed products. Where public or international resources are proposed to be applied, however, some vision should be gained of how such scarce resources should be most suitably applied in order to respond to the most pressing scientific and humane needs.

Clinical trials

Choice of prospective populations

Initial identification of the population from which research subjects will be sought is governed by the scientific demands of the individual research protocol. Often, however, alternative populations will serve the scientific agenda equally well. A selection should then be made that is sensitive to the different ethical implications of choice. For instance, study risks may be greater with an undernourished population than with an adequately nourished population, suggesting that the latter be favoured. Equally, however, it may be just to provide scarce resources to favour an impoverished population over one that is more affluent. A dilemma exists where a malnourished population is also impoverished in contrast to a better nourished population. Similarly, reproductive research may more safely be centred on women in the middle of their reproductive years because of the added risks of pregnancy in the old and the young. A marketed product not tested on women at each end of the spectrum of reproductive age may either expose them to unknown risks, or not be prescribed for them. Because of extra risk in pregnancy, they may be recipients of greater benefits from development of a safe product, rendering it appropriate that, as greater potential beneficiaries, they bear at least the same risk in the conduct of research as women in the middle reproductive years.

Randomized clinical trials

Differently structured studies may be presented as randomized clinical trials (RCT). The key feature of such studies is that subjects are allocated to treatments in accordance with a mathematical or scientific formula and not by their own choice exercised after receipt of their physician's advice directed to their best interests. It should be made clear to subjects that clinical judgment directed to their welfare is suspended for the purposes of the study. Even when subjects are to receive one randomly assigned orthodox treatment before being switched to another orthodox treatment with which a comparison is to be made, they must be informed that they are being treated as subjects and not ordinary patients. Where an orthodox treatment is being contrasted with a new or an unorthodox treatment, and where such a treatment or an orthodox treatment is being contrasted to no treatment when treatment is medically indicated, each subject must be informed of the nature of the comparison and that there is a chance of not receiving any treatment when treatment is medically indicated. Subjects must similarly be informed when the study design involves taking placebos that mask whether or not an active substance is being taken.[43]

Multicentred trials

When a study population is larger than a single facility can supply, or when required variables can be found only by recourse to different populations, a

study may be conducted through collaboration of different research centres. The study should be ethically acceptable in each of the different centres where it is to be conducted, and no centre should be asked or expected to compromise important ethical principles for the sake of collaboration. Occasionally, however, a centre may tolerate departure from its customary ethical requirements in order that the study proceed, when without collaboration it could not be satisfactorily conducted or completed. The institutional ethics committee may invoke macroethical reasons to justify departure from principles it routinely applies in microethical assessments. A discrete set of ethical standards is applicable to multicentred studies, including that there be a central co-ordinating committee responsible for gathering relevant documents and data and for distributing them as appropriate to collaborating centres.

Informed and free consent

Governing principles of informed consent that have been seen above in the setting of legal issues are an expression of values having their origins in ethics. It has also been seen, however, that the ethical significance given to autonomy is not universal, and that sizeable populations of the world are inspired by principles of interdependency and communality that may be contrasted with individual autonomy. An ethical problem arises when research protocols established or influenced by cultures that emphasize autonomy are proposed to be implemented in interdependent or communal cultures.[44] A danger of such protocols that exceeds their medical risks is that they do violence to the cultures in which they are to be implemented, or that they may irreversibly change them. Equally, investigators who operate outside their own countries may suffer accusations of violating abroad the principles of autonomy that they are required to observe at home. To export domestic ethical requirements raises the charge of ethical imperialism, while investigators' invocation of principles of cultural relativity that justify behaviour abroad that is not permissible at home leaves them accusable of violation of international minimum standards of behaviour that are universal.

Regarding fertility-regulating vaccines, research is liable to be conducted on women in male-dominated cultures where consent includes that of social leaders who are male. Where women are socialized to be obedient to men and not to manifest independent will or judgment, women may be inhibited from declining to participate in studies that social leaders have found to be beneficial for the community. To approach such women before receiving approval from their social leaders might be bewildering to them, disrespectful or threatening to the leaders, and potentially subversive of the prevailing culture in indicating to the women that they are expected and able to make decisions autonomously.

Reproductive research is particulary sensitive where it involves unmarried sexually active adolescents. Parents may deny or fear, particularly, their

daughters' sexual expression of affection, and believe that the offer of contraceptive protection or other prevention of childbearing will induce them to become sexually promiscuous and careless. Where adolescents are mature enough to exercise informed choice, they may be invited to act as research subjects, but investigators must exercise caution to pay adequate respect to parental sentiments not to jeopardize daughters' positions within their homes and not to aggravate or appear to exploit adolescent rebellion or disdain for parental values.

Confidentiality

The ethical value of confidentiality expected to be shown in medical care and medical research may be of special significance where individual regulation of fertility is concerned. The two special ethical challenges that confidentiality poses are, first, that confidentiality may amount to subversive secrecy in the settings last considered, and, second, that confidentiality may preclude from the knowledge to which they feel entitled parents of sexually active adolescents and husbands who want their wives to bear them children. Responses to these challenges must be informed by governing laws and sensitive to social values.

Community involvement

Although a community representative may sit on an ethical review committee of an institution, public providers of research means may feel an ethical obligation to inform the communities they aspire to serve of the sort of research they sponsor, facilitate or accommodate. The level of generality at which information is pitched is initially a matter of responsible judgment, but information should not be given in a way that may jeopardize confidentiality or lead to prejudicial misunderstanding about the nature or conduct of the research in question. News media and the wider public may be afforded means of understanding the needs, methods, potential risks and intended benefits of research proposals; risks should not be unduly downplayed nor possible benefits exaggerated.

Marketing and use

National criteria for marketing approval will be based primarily on macroethical considerations. Determinations of policy tend to be expressed and implemented through law, but because ethical needs or excuses may become apparent that are not accommodated by generalized legal principles, legislation and courts will frequently permit exceptions from general rules. Compassionate exceptions may afford individuals access to products not available for general use, as may exceptions for the conduct of research. In this area, as in many others where government operates through laws, it must be remembered that law is the servant and not the master of ethical judgment.

Product advertizing

Advertizing of products available only on prescription is normally confined to the medical profession, but generic advertizing may occur when items about a medical development appear, perhaps following trade encouragement, in popular news media. It is ethical in principle to give information of products, and to promote them in view of the benefits they are conscientiously claimed to offer consumers. Principles of respect for persons require, however, that no deception be used, deliberately or inadvertently, such as by suppression of rates of product failure or of adverse findings or risks. Further, protection of the vulnerable precludes advertizing designed to attract consumers desperate for cures or relief that exceed what the product has been proven capable of achieving. The forms of advertizing used, including the placement, visibility and, for instance, timing of advertizements, are also matters for which manufacturers and others in the chain of supply to ultimate consumers are ethically responsible. Engagement of advertizing agencies offers relief from ethical responsibility only to the extent that an agency enjoys a reputation for ethical conduct and to the extent that its advertizing plans are honestly and comprehensibly presented. Wilful blindness to unethical advertizing plans constitutes complicity in unethical conduct.

Consumer counselling

Consumer counselling is facilitated by general public education, but is often dependent on specific informing and training of medical and other health personnel. It is not clear that a supply of fertility-regulating vaccines raises a distinctive set of ethical issues beyond those involved in the supply of comparable contraceptive, contragestive and abortifacient products, except, perhaps, that the principle of beneficence requires that counsellors be given up-to-date information about their relative effectiveness and safety, and that there be speedy channels of communication of results of continuing research.

Compensation for injury

The sense of ethical obligation to provide for compensation of those who suffer vaccine-related injury is expressed in the legislated schemes that have implemented compensation programmes. Such programmes may reflect commitment to a philosophy or ideology that is not necessarily universal, and may alternatively be explained by utilitarian or instrumental goals to encourage development of products considered generally beneficial by protecting manufacturers. This may be less the case where manufacturers themselves are required to provide the compensation fund from profits, but where government supplies the fund this may constitute a subsidy for product manufacturers who are relieved of the need to acquire commercial insurance or to invest in self-insurance. Different philosophies produce different beliefs regarding whether and how compensation should be provided for those who suffer vaccine-related injuries. Courts of law may identify a prevailing

philosophy in rendering judgments in negligence or product liability claims, but it may be fitting that responsible agencies of government and within the product manufacturing industry determine and express what they consider the appropriate ethical response to be.

International implications

Legal principles of domestic and international law that have been considered above with regard to legal issues fit into a broader ethical setting. Values material to both macroethical and megaethical analysis may be at stake in the terms on which countries conduct their international relations and the transfer of biotechnology. This is more acutely the case where population development is concerned, because of perceived connections between individual fertility, population growth, nutrition and housing of human communities, urban growth and the danger to natural habitations of urbanization that depletes rain forests, wetlands and other natural means of sustaining and replenishing the environment.

Decisions regarding national acceptability of fertility-regulating vaccines respond to factors that, in general terms, may be separated into the objective and subjective. Objective factors are scientifically determined, and include effectiveness of vaccines on studied populations, and related side-effects. Populations may react differently to the same product because of, for example, genetic, dietary and environmental reasons, but the consequences of use of the product are objectively verifiable and replicable. In contrast, subjective factors are judgmental responses to both objective and intuitive perceptions regarding whether a product is suitable for a particular population. Whether the interaction of the proven side-effects, effectiveness rates and dysfunctional consequences of a product warrant its availability for a given population is a question of policy. The policy expresses subjective assessments of policy makers and not a demonstrable calculus.[45] The levels of efficacy and safety that are set for a product to be acceptable are subjective and influenced by values, including ethical values.

The values that affect approval of fertility-regulating vaccines for a domestic population, including approving importation, are not necessarily the same as those affecting exportation. What products are developed within a country will be strongly influenced by factors derived from the country's socio-economic system. Governments clearly have less control in free-market economies than in state-run economies, although both may be receptive to initiatives originating in international institutions such as the World Health Organization.

Once a product is developed within a country, however, its exportation involves governmental control. That is, even countries with free-market economies control technology transfers and exportation of pharmaceutical and biological products. The principle of respect for persons is projected on

the international plane, where autonomy is translated as sovereignty. Beneficence consists in the good will and good faith that countries are expected to apply in their international dealings, reinforcing the protective initiatives ethically required to protect vulnerable populations, including those vulnerable because of lack of means of fertility control and birth-regulating options available to those who wish to use them. Justice requires not necessarily that each country have identical resources, but that health resources be available to permit all human populations to enjoy at least a common minimum standard of physical, mental and social well-being. While not every country can discharge the same responsibility to furnish resources to others, no country should obstruct exportation of surplus health resources to others that are willing to import them. Capacity to import by purchase depends on economic means, but protection of vulnerable populations may require subsidy or assistance programmes based on national and international initiatives to afford poor countries the means to import health products for which they assess they have a need.

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14 Social aspects related to the introduction of a birth control vaccine

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Introduction

The aim of this chapter is to discuss potential user attitudes and reactions to birth control vaccines. Several such vaccines are under development, including antisperm and anti-ovum vaccines which will be effective prior to fertilization, as well as anti-hCG and antitrophoblast vaccines which will be effective after fertilization. Although user attitudes and reactions will be different for each of these vaccines, this chapter will focus on the social aspects of the introduction of an anti-hCG vaccine as this is likely to be the first to enter large-scale clinical use.

As the anti-hCG vaccine is still in a developmental stage, the discussion is constrained by a hypothetical situation and will, therefore, be largely speculative in nature. The introduction of the birth control vaccine on a broad scale in developing countries will not occur until some time in the future, but it will be in these countries where the problems and challenges posed by this new contraceptive option will be most numerous and complex. Other issues may arise in industrialized countries which normally have very effective drug-regulatory agencies and health-delivery systems.

At this stage there is naturally no literature on user reactions and experiences with this form of contraception. The discussion will therefore draw mainly on social science research relating to injection as a route of administration and to general experiences with other types of vaccines currently in use in immunization programmes.

Description of the vaccine

Present biomedical research indicates that, in the future, one injection of the birth control vaccine could provide between 12 and 24 months of protection against pregnancy. Women would not need to take any other action during this period. There is no need to take a pill every day or to visit distant facilities to get a booster injection during this time interval. Furthermore, it seems that the vaccine may have fewer side-effects than many of the other hormonal or intrauterine contraceptives now in use. Side-effects have been shown to be an important reason for discontinuing use of existing injectable methods.[1] The

fact that the vaccine is administered by an injection has also been emphasized as an easy form of delivery which could be conducted by paramedical personnel and which therefore will increase the vaccine's availability.

An additional advantage of the antifertility vaccines currently being developed is that they can be prepared and distributed in a stable, dry powder form and will have, therefore, a long shelf-life under a variety of storage conditions. Unlike some other vaccines, they will not be sensitive to changes in temperature and will not require a cold chain system to ensure their effectiveness at the final delivery point. The chances of administering these vaccines to women in an ineffective or damaged form are thus diminished.

However, there are also some important disadvantages from a user point of view. The most important is that the vaccine, unlike other long-acting methods such as steroid-releasing implants, is non-reversible. In other words, the potential user cannot discontinue use of the method if she decides to become pregnant or is experiencing a real or perceived health problem. Once the injection has been given, the effect will last for 1 to 2 years. Another disadvantage is that there are no outward signs to show when the vaccine stops being effective. Although the need for a simple self-administered test for verification of protection has been discussed, there is currently no means by which a woman can tell whether she is still protected by the vaccine. A consequent potential danger is that a woman might receive a booster injection during the early stages of an undetected pregnancy or whilst lactating. Although studies are underway in this area, the consequences for the mother, fetus or infant if this occurred are still unknown. In order to reduce such risks, providers should be able to inform women about the maximum period between booster injections. This issue might argue against a more widespread delivery in non-clinic settings or by paramedical staff, at least initially.

While the ease of administration, by injection, has been emphasized as one of the main advantages of the birth control vaccine, this method also carries some health risks. It is well known that equipment sterilization procedures in many developing countries often leave much to be desired. A contaminated needle can transmit serious diseases such as AIDS and hepatitis. Great care must be taken to ensure that the equipment is always sterilized or that the vaccine is only administered by a system that is sufficiently cheap to enable it to be thrown away after a single use, thereby greatly reducing the risk of re-use. Logistics related to storage, doses, and records require careful attention to avoid confusing the vaccine with other injectable preparations.

It may be necessary to identify women whose genetic background would cause them to be non-responders to the vaccine. Although this might be determined by a simple blood test, more costly, sophisticated tests may be needed that require facilities, knowledge, equipment or time that are not available in developing countries. Likewise, some women may experience allergic reactions to the vaccine. However, the incidence and severity of this and other side-effects will be thoroughly investigated during clinical trials.

Table 14.1. *Advantages and disadvantages of a birth control vaccine from the user's viewpoint*

Advantages	Disadvantages
Long-term effective contraceptive protection	Non-reversible for 12-24 months; cannot be stopped by user
Few side-effects; regular menstrual periods	Uncertainty about when effect stops; risk of pregnancy
Ease of use; no user actions necessary; coitus independent	Potential risks if vaccinated while pregnant
Increased privacy; no visible signs of vaccine	Duration of effect may vary according to age
	Potential risk of interaction with other vaccines.

Table 14.1 illustrates the advantages and disadvantages of the vaccine as seen from a user's point of view. Each of these characteristics will be discussed in the following pages.

Women and the context of reality

The subject of this chapter is the introduction of a new birth control vaccine among women who have already decided to use some form of family planning. But it is important to remember that the factors influencing this decision may be complex and vary from one cultural setting to another, and particularly from the clinical trial situation determined by biomedical research norms. The sociocultural background of women determines the way they perceive their options in terms of family planning and, therefore, also their perception of the advantages and disadvantages of the new birth control vaccine. The context of reality for women in developing countries includes religious and cultural aspects, social and economic relations/roles, family formation stages and marital statuses, political priorities (on the national as well as local level) and the characteristics of the public and private health delivery systems. Each of these factors must be taken into consideration when discussing possible user reactions to the birth control vaccine.

Cultural considerations

There are many meanings, interpretations and values surrounding the concept of family planning and these vary throughout the extensive cultural variety of the developing world. Limiting the number of children may go against the woman's religion or her husband's expressed desires. In many societies, a large number of children is perceived to be an asset and a security in old age. The fact that the birth control vaccine is non-reversible during its intended duration of effect may lead to feelings of insecurity or worry if another pregnancy is desired at some point in the near future. An additional concern is that women

may receive a booster injection while already pregnant because they are unaware when the vaccine stops being effective. A few tragic cases of abortions or deformed children would have a rapid negative effect on the vaccine's acceptability and possibly limit its use to very special circumstances.

If the duration of efficacy provided by the birth control vaccine is found to vary greatly from one woman to another, the vaccine may be perceived as unpredictable or unreliable. This variability may even cause fears about permanent infertility. It will be extremely important to screen and counsel women carefully, especially older acceptors, about when they will regain their fertility. An important issue with respect to the return of fertility is related to the ability of people to keep accurate track of time. This is a problem in many developing countries, especially in rural areas, where time durations set in large segments – 18 months, for example – may be hard to remember. Some mechanism will have to be set up to make sure women know when the vaccine effect stops. Service personnel will probably have to consider some careful follow-up procedures to remind their clients of the need to return to verify their fertility status.

On the other hand, some of the positive characteristics of the vaccine may make it an attractive alternative for women who do wish to employ some form of long-term protection. An important advantage is that the vaccine does not cause changes in the menstrual pattern and this will probably contribute to its popularity. For example, Jamaican women were worried about using Depo-Provera because they went for months or years without menstrual bleeding. Menstruation is perceived as bodily cleansing, a sign of good health. The lack of menstruation gave rise to fears about permanent loss of fertility.[2] Other studies have confirmed that irregular or prolonged menstruation can be a source of concern for women, especially in cultures where women are considered ritually unclean, and therefore restricted, when bleeding. The vaccine's apparent lack of this type of side-effect will probably make it an attractive family planning option in these cultures.

The mode of administration, by injection, is also likely to contribute to the acceptance and use of the birth control vaccine. Injections have proved to be very popular in many developing countries.[3–7] There seems to be a widespread belief that medicine given in the form of an injection is more effective and more powerful than any other type or form of medicine, irrespective of what is actually in the syringe. The local irritation which the intramuscular injection with the birth control vaccine may cause will probably be perceived by its users as a sign of its efficacy.[8] Previous experience with curative or preventive injections may add to the popularity of the vaccine: for instance, yaws eradication campaigns where a single injection with penicillin achieved spectacular results in a very short time[4,5] In some cultures, research has revealed that injectable contraceptives were the preferred form of family planning,[9] although these injectables were never as long lasting as the new birth control vaccine, being limited to 1, 2, or 3 months' effective duration.

It is essential that cultural attitudes to injections are known before the vaccine is introduced in some settings. Sometimes injections are perceived to be inappropriate for older people because they are believed to be very powerful.[10] In other cultures, a vaccination for a child may be perceived to be of cold substance resulting in a hot compress being put on the immunization site by a relative. Such a hot compress can cause a swelling which might compromise the effects of the immunization.[11] Naturally,the birth control vaccine will have greater acceptance in countries where injections are popular and widely accepted as a good way to administer medicines.

In summary, the vaccine is likely to lead to the following advantages, but is apt to pose also certain disadvantages.

Increased popularity ⟨—— Administered by injection ——⟩ Health risk
Increased availability ⟨—— Easy to administer ——⟩ Risk of abuse
Sign of efficacy ⟨—— Local irritation ——⟩ User discomfort

Social and economic factors

Gender roles are changing in many developing countries. In some cases this has meant increased opportunities and decision-making power for women. But in many cultures, decisions about a woman's reproductive capacity are not taken by the woman alone. Husbands, mothers-in-law and other important members of the extended family may have different ideas about the desirable number and timing of children. Sometimes, husbands are opposed to their wives' use of any contraceptive method because of the perceived effect on women's fidelity.[12] In this respect, the birth control vaccine offers some advantages. Since there is no pill to take daily nor a visible implant under the skin, the vaccine allows the woman a privacy of choice which she may not have with other methods. Of course, this is also the case with injectable steroids, but in this instance the necessity of returning for more injections every 1 to 3 months increases exposure to public scrutiny by neighbours or relatives.

Some of the literature on other vaccines* has stressed that it is often groups of low socio-economic status which are associated with low immunization rates.[13] One of the suggested explanations has been that these groups would have more traditionally oriented ideas which would prevent them from using allopathic services.[14] However, numerous studies have shown that most people are pragmatic and will use both traditional and allopathic health care, depending on which is perceived by them to be appropriate.[15–17] What is of relevance here is that the mother's level of education has been seen to be related to the acceptability of child vaccinations. But '. . . it is not the content of

* A certain degree of caution is necessary when referring to literature on immunization. When the goal is immunization against childhood diseases, coverage becomes very important and cultural and social inhibitions may be seen as obstacles to be overcome. In the case of antifertility vaccines, however, cultural and social factors should not be regarded as obstacles but should, in fact, be respected.

what the educated mother learns at school that is so important, but rather the associated changes in her attitude to, and position in the world . . .'.[18] One may assume that this would apply to the birth control vaccine as well. An educated, self-confident woman would probably be more aware of possibilities such as the new vaccine but she would also be more critical and more outspoken about possible side-effects and disadvantages and more ready to demand alternatives from providers if necessary. She would also be better able to deal with the time elements involved and, consequently, with the return of fertility.

Another experience from research on acceptance of immunization may also apply to the birth control vaccine: namely, the effect of mobility and isolation. Low integration in the community and limited social networks may diminish women's ability to obtain information. Seasonal and urban migration may adversely affect not only knowledge about family planning possibilities but also opportunities to remain protected, i.e to receive the next injection at the right time. Furthermore, immunization rates have been shown to be higher among families where the mother is a member of a social group, whether the reason is transfer of information or social pressure.[13] It would be interesting to investigate if social integration affects the acceptance and use of the birth control vaccine as well.

Economic factors may also be important for the future acceptability of the birth control vaccine. To make the vaccine a realistic option for women, it must be of low cost or free. Because their incomes are very limited, the majority of people in developing countries can only purchase small amounts of allopathic drugs at a time. While women may be able to afford costs spread over a period of 12 months, this does not mean that they could pay the total amount once every 12 months. Poor families often have to survive by a sort of crisis management from day to day which makes it very difficult, if not impossible, to save money. In a fee-for-service system, therefore, obtaining the birth control vaccine should not require a relatively large financial expenditure, even once a year. If the delivery system is such that poor women can obtain family planning services for free, care should be taken that documenting poverty in order to receive a free injection does not result in difficult or humiliating situations for women.[19]

The relatively long-lasting effect of the birth control vaccine does enable women to save time, more so than with many other methods. Instead of having to visit the family planning facility or pharmacy every 3 or 6 months, there is a span of more than a year between the necessary injections. If the facility is far away or the woman has difficulty being away from children or work, the time- and cost-saving aspects of the vaccine may be important advantages.

Political factors

Local authority structures (community leaders, headmen, etc.) can be instrumental in persuading people to use preventive services, e.g. vaccinations,

to the benefit of the population as a whole.[13,20] Unfortunately, there have also been examples of political abuse, and the history of family planning is a case in point. Sterilization and contraception have been forced upon vulnerable groups who for various reasons (ethnic, cultural, religious, economic) were not favoured by powerful decision-groups, whether national or local. Overzealous health workers whose salaries depended on the success of birth control methods in their areas have been reported to compel or trick women and men into unwanted contraception. The consequences for the individuals involved and for the cause of informed and voluntary family planning have been detrimental and tragic.

It is crucial that the birth control vaccine is not abused in this way. The fact that it is administered by an injection makes it easy to confuse, intentionally or unintentionally, with other preventive and curative injections. Almost anybody can administer an injection. The advantage of being long lasting will be a problem instead of an advantage if the vaccine is given without the woman's informed consent in that the client's control, over whether or not to comply with the professional's advice, will have been eliminated.

International and national family planning agencies must collaborate with groups (religious, women's, consumer organizations) at the grass roots level to ensure that the birth control vaccine is always offered as a safe, voluntary option, and that women have adequate and reliable information with which to make their own choice.

Given the fact that this is a provider-dependent method and that it can be easily administered, there is a danger that it can be used in subtle yet effective ways to 'control' a woman's fertility without her full knowledge of what is happening. It would be advisable to use another name to introduce this method into programmes rather than 'birth control vaccine', such as antifertility vaccine or fertility-regulating vaccine.

The service delivery systems

The service delivery system for family planning varies widely from country to country. Sometimes family planning services are integrated in the general health care system, sometimes they constitute a separately functioning programme. In most cultures there are private alternatives, with varying degrees of legality, to the public service delivery system. A distinction between the public and private sector is often artificial because manpower as well as supplies (drugs, syringes, etc.) tend to flow from the former to the latter.[21] It is more than possible that this could happen with the birth control vaccine as well. It is important, therefore, to discuss not only the public delivery system but also the potential consequences of the vaccine being adopted and adapted by the private sector.

The public service delivery system

The public service delivery system of family planning is not necessarily entirely government financed but it is, at least to some extent, government controlled. In many developing countries, health ministries have insufficient funds to provide adequate health care and family planning services to the population. Even with outside assistance from multilateral or bilateral donors, the situation is often less than satisfactory. One consequence of this situation is that government personnel frequently are insufficiently trained and paid and, therefore, not always motivated to spend scarce time advising clients nor to make additional efforts to follow them up.

As with other family planning methods, it is crucial that women are given reliable information, in a readily understood form, about the effects, mechanism of action and alternatives to the birth control vaccine in order to allow them to make an informed choice. It is equally important that the method selected is delivered in a safe way. For these conditions to be fulfilled, the providers need to be trained in how to counsel women on the advantages and disadvantages of the birth control vaccine. It is also important that the staff are sensitive to the cultural and social context of such women and that they treat women from different status groups in the same professional way. In some societies, it will be essential to ensure that the vaccine is given in absolute privacy, allowing the woman to keep her decision secret.[12] In other societies the support of local authorities may determine the extent to which vaccinations are accepted.[20] In each culture and subculture, the responsible personnel must be aware of the cultural decision-making process with regard to women's fertility and act accordingly to ensure and facilitate a free choice.

Family planning personnel must also have the necessary medical training to make sure that the vaccine is given safely. The provider must be certain that the woman is not pregnant before the vaccine is administered. The importance of sterilizing re-usable syringes and needles must be borne in mind. Such requirements may exclude certain categories of paramedical personnel from administering the birth control vaccine. Ideally, the personnel should also have the training and equipment to carry out a simple screening of women before advising them on their suitability to receive a birth control vaccine. However, this does not seem to be a realistic possibility in a developing country context, in the immediate future.

More than anything else, over-zealousness on the part of the family planning staff to coerce women to use the birth control vaccine must be avoided. The fact that the vaccine is so easy and quick to administer makes misuse of it a potential danger. Abuse of the birth control vaccine would not only harm family planning in general but it could also have negative consequences for public attitudes to other vaccines and to the health care system in general. Health care planners may wish to consider a special service delivery structure for the future introduction of the birth control vaccine. If

possible, a structure should be considered in which personnel are not rewarded for the number of women to whom they administer the vaccine, but rather for the quality of information they provide to potential and actual users.

The private sector

The private sector comprises many different types of health care providers, some legal and some illegal. Not only qualified, private doctors prescribe and dispense powerful allopathic drugs. Traditional healers are increasingly using allopathic drugs in their practice[23,24] and people with no or minimal medical training set themselves up as travelling drug peddlers or as 'injection doctors'.[3,21] This type of informal practitioner profits from a widespread demand for allopathic drugs and especially injections. By bridging the status-gap between formal health care providers and lay people, these providers become an attractive alternative to the public service delivery system.

It can be anticipated that in one way or another the birth control vaccine will eventually also become part of the informal providers' treatment repertoire. The vaccine will be especially lucrative for these practitioners because it is administered by an injection, a route of administration which is very popular in developing countries and for which clients are prepared to pay more. Furthermore, the vaccine's fertility-regulating purpose calls for a privacy often more easily obtained by the client from an 'injection doctor' than from a crowded waiting room of a public family planning clinic.

While the birth control vaccine's adoption by informal, private providers may increase its availability, it may also carry serious health risks. Procedures for the sterilization of equipment are either grossly insufficient or totally lacking.[4,22] Syringes and needles are used over and over again. Possibilities for screening or pregnancy tests are totally non-existent. Women will probably receive information about different aspects of the birth control vaccine but this will be very different from what is considered optimal by health or regulatory agencies.

Unfortunately, it is also likely that attempts will be made to use the birth control vaccine as an abortifacient. User perceptions of the way contraceptives work have in some cases led women to believe that if one pill a day prevents pregnancy then a lot of pills taken at once will induce an abortion.[12] Reports from developing countries have pointed out that sometimes hormonal drugs are used as abortifacients. The fact that the vaccine is administered by an injection will probably add to the general perception of its multipotency. Its perceived potential for inducing abortions will no doubt be exploited in some developing country settings.

Poor or inadequate drug-regulatory capacity, lack of control of drug supplies coupled with the innovativeness of multiple 'health entrepreneurs' in developing countries, must be borne in mind when the birth control vaccine is introduced. Some of the very obvious advantages of this vaccine may result in

serious health risks if health care planners do not foresee the dangers of an uncontrolled use in the public and private sectors.

Needed information and potential users

Given what is known about the birth control vaccine today (the apparent lack of side-effects), the most important information in terms of the potential user seems to be reliable advice on when fertility will return. Providers would have to be able to answer questions such as the following.

> How long after an injection does it take before fertility returns? (The woman's age may influence this.)
> How does a woman know that she is no longer protected?
> When should a woman return for the next injection?
> What happens if the woman is already pregnant when she receives the injection?
> Does the vaccine interfere with breastfeeding?
> Does the vaccine interfere with the action of other vaccines or drugs?
> Why is it important that only qualified providers administer the vaccine?

The potential users should be encouraged to report any side-effects, irrespective of their significance in a biomedical sense, as well as opinions about the way the vaccine was administered to them. Adequate counselling should be available to vaccine users at all stages.

At the present stage, it is very difficult to speculate about the types of women who would be likely to use the birth control vaccine. Much will depend on the particular culture in question and the way this new family planning option is presented to them. One hypothesis could be that married women who have already had the number of children they want would be attracted by the time-saving and privacy aspects of the vaccine. This type of user would often be an older woman, but cultural attitudes towards the appropriateness of injections for older people may influence this situation. The privacy aspects of the vaccine may also attract younger women who may want to space or limit the number of their children with or without the consent of other family members. For such women it would be especially important to give reliable information about when fertility will return. Furthermore, the long effect of the vaccine may seem especially worthwhile for rural women who often have to travel long distances to family planning facilities, who have limited time at their disposal, and who do not have access to a convenient transport system.

The types of women who are likely to use the vaccine will probably vary from culture to culture. The previously mentioned privacy aspects will be very attractive for a broad range of women in societies where women have little individual decision-making capacity, including control over their own fertility. The vaccine's mode of administration, by injection, will in many cultures contribute to the method's popularity. It should be stressed again,

however, that the way a product like the birth control vaccine is presented and administered to potential users is at least as important as the characteristics of the product itself. If women lack confidence in the service delivery system or the competence and motives of the personnel involved, then the potential benefits of the new birth control vaccine will be misunderstood and wasted. It is therefore of utmost importance that acceptability research takes into consideration the sociocultural reality of potential users as well as the way information about, and administration of, the vaccine are perceived by women in developing countries.

Suggested research priorities

As with any new method that is assessed as it becomes available for public use, social science research would complement biomedical studies and clinical introductory trials by providing information from the perspective of the user, or potential user, of the vaccine. The principal aim of social science research during the process of contraceptive introduction is to identify the main determinants of acceptance and successful use of new technologies. The following list includes some of the issues that would receive high priority for study once the vaccine has become available.

- Which positive and negative characteristics of the vaccine are most important to users and why?
  Which characteristics of the vaccine are found to be most beneficial and which the least beneficial?
  What side-effects, if any, are perceived and how do these perceptions relate to broader health beliefs?
  How would these characteristics of the vaccine influence the potential users' decision to avail themselves of the vaccine and, once started, continue use of this method?
  What views do users have of the service delivery system and how do these views ensure or impair satisfaction with the use of the vaccine?
  How would these views influence the decision to continue using the vaccine once its effect has expired?
  What are the best mechanisms for ensuring an effective follow-up of users and to establish absolute evidence of return to fertility in a manner that is satisfactory to users?
  What kind of information would potential users like to receive and what additional information should be given to acceptors during use?
  What types of service mechanisms would work best, both in terms of user assurances that the effect of the vaccine has ended or that they are non-pregnant and thus able to receive a second vaccination?

The list is already long, but as the method becomes available it may grow even longer as questions arise that are very specific to certain service delivery systems and specific to a method that is provider-dependent and non-

reversible during the specified period of efficacy. Given these two key factors, ensuring user satisfaction will require careful study of the social forces that come into play, particularly in the early stages of the vaccine's introduction into family planning programmes. Special attention will have to be given to foolproof service procedures that eliminate the possibility of administering the vaccine to a pregnant woman. Such concerns call for avoiding, at least during the early stages of programme introduction, vaccination by community health workers or other paramedical personnel who cannot provide potential users with the required assurance that they need to receive the vaccine. Similarly, care will have to be taken that information given to users makes it clear, beyond any doubt, that the vaccine is not, or cannot be used as, an abortifacient.

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15 Chairman's summary: Present status and future prospects of antifertility vaccines

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1989 is the right year to hold this meeting. Phase I clinical trials of three human chorionic gonadotrophin (hCG) vaccines are approaching completion and all have been found to perform well. If not quite the first such vaccines ever, they are certainly by far the best studied and the most hopeful. In these trials, the vaccines have been tested only for their safety and their ability to elicit antibodies, but not for their ability to reduce fertility, and so we do not yet know whether they will manage to work in the way intended. Their efficacy will be tested in future clinical trials. Nevertheless, the success of these trials gives us reasonable grounds for hope that a vaccine of this type will indeed act as a safe, long-term antifertility agent. The faith and perseverance of the scientists who developed these vaccines have been justified, and the world owes a debt of gratitude to them and to the women who had the courage and altruism to volunteer for the trials.

That having been said, it is appropriate to ask why it has taken so long to reach the present stage of development of these vaccines. Roger Short tells me that it is just 20 years ago that, independently, several investigators embarked on this type of research, and there are two very good reasons why that time has been needed. The first is that we are dealing with two of the most complex parts of the body, the immune system and the endocrine system. We are exploring the interaction between them, and the difficulties which that presents have been vividly illustrated in various ways during the discussions at this symposium. For example, we still do not know exactly how immunization against hCG impairs fertility, and it may be a long time before we do so, because even with the best and oldest-established anti-disease vaccines, the exact mode of action is often still not understood. It was reflected in another way when some of the participants in this symposium asked why is there no booster effect when exogenous whole hCG is administered to the hCG-immunized women. At least four different explanations were offered, none of which commanded full confidence.

In attempting to explain why the immune and endocrine systems are so complex, various reasons come to mind. Francis Crick has drawn attention to the fact that natural selection is a great tinkerer. When natural selection finds something that works as well as the immune system does, it starts to try out variations. Some survive, some do not. To understand how the immune system or the brain or the endocrine system works is very different from asking

an engineer to design them *de novo*. He would adopt a more simple and straightforward approach, but probably not a better one.

As a further complication, the immune system uses a set of overlapping genetic polymorphisms, such as occur in human lymphocyte antigen (HLA), the complement system and in the antibodies themselves, in order to resist infection. The pathogens which beset us evolve extremely rapidly, so the immune system has chosen to retain the flexibility of polymorphism in order to cope with these constantly changing challenges. We inhabit an evolutionary circle that goes on building variation, and it is that which has made the task of the vaccinologists so difficult.

At this time, it is appropriate to look forward on three issues. First, what further steps will be needed in order to bring the hCG vaccines through to the stage of general use, if they turn out to work as well as we hope? Bringing a vaccine into use is not a simple matter, for it will involve a very great deal of safety testing, evaluation for safety and acceptability, and the identification of suitable groups of women to be offered the vaccines, particularly in the early stages of their clinical assessment. This will require far more effort than would be appropriate for conventional anti-disease vaccines, simply because a successful antifertility vaccine is likely to be used more often and more widely than any existing vaccine. In addition, an antifertility vaccine would need to be equivalent, in terms of risk–benefit, to established family planning methods, and will need to pass safety tests of the utmost stringency. The fact that three rather similar hCG vaccines have come forward at the same time presents an interesting situation. It is a great advantage that their development should move forward in parallel, so that claims can be tested independently and diverse possibilities explored more rapidly. However, the three vaccines should not be perceived as rivals as clinical trials might well disclose that each member of this group of vaccines may be suitable for a particular use. WHO has an important role in fostering co-ordinated trials of the different vaccines, so that the optimum preparation can be designated for use in particular circumstances and in order that women should not be put through the inconvenience or risk of redundant trials.

The second issue on which we can look forward is the development of additional birth control vaccines. The background papers and report of this symposium survey the possibilities available to us in this area. One broad consideration that has guided this survey is that, thanks to progress in the cell biology of reproduction, a growing number of cells and cell-products can be identified as vaccine candidates. We might attempt to immunize against other hormones besides hCG, for instance, or against gametes or their products, or against the trophoblast. In fact a search for the best components of such a vaccine has already started, as the papers presented indicate. We expect that the final outcome may be a composite vaccine, which aims at more than one target, and thus (a) ensures effective contraception, and (b) minimizes the immunological activity required of each individual component.

In the development of these new vaccines, we recognize the power of molecular biology. Progress in that science has enabled many molecules that would otherwise be inaccessible to be considered for use in vaccines. This is partly because molecular biology provides powerful screening methods for examing an enormous range of proteins, and partly because proteins which occur infrequently in the body can now be manufactured in bulk by biotechnology. Thus, for instance, one can hope to explore the potential of every single protein expressed on the surface of spermatozoa. Furthermore, as the symposium report mentions, it is likely that these possibilities will open up for carbohydrates, as well as for proteins, so that important but hitherto inaccessible parts of the reproductive system, such as the zona pellucida, will become available for use in vaccines.

Another consideration in the search for new vaccines is the growing availability of new immunological procedures. These include a series of developments in vaccine delivery systems such as microencapsulation, which do not introduce new principles but which nevertheless greatly extend the range of usable antigens and the duration of protection that they may produce. More radically new is the possibility raised here of introducing a T-cell vaccine, for instance against the trophoblast, that would work through the action of effector T-cells rather than through antibodies.

The third issue on which we can look forward is the consequences of bringing antifertility vaccines into general use. There are deep questions that concern national population policies, the role of WHO, the position of ethics, forseeable abuse, national priorities, and the balance between individual rights and the needs of future generations. This survey does little more than touch on these matters, quite properly perhaps at a time when we do not know whether a safe, effective and acceptable antifertility vaccine will ever become available.

Our discussions revealed that an antifertility vaccine can be regarded from sharply contrasting points of view. Some see it as little more than an extension of existing methods of medium-term contraception, such as the Norplant; others feel that the potential for ease of administration, coupled with cheapness and long-term effect place it in an entirely new category. Some regard it as tampering with germ cells and the complexities of the immune system, and therefore extremely dangerous; others see it as safer than steroids, because it immediately stops something from happening, rather than continually blocking a normal activity. Some hope for a vaccine simply because it will provide women with a worthwhile new birth control option, while others hope that it will provide an unprecedented effective instrument of demographic control.

Concerns were expressed about the inability of a vaccinated woman to recover fertility at will, her difficulty in knowing when fertility returned, and the possibility of vaccination to procure abortion. To an extent, these concerns were resolved in the course of discussion, but the attention of the reader is

drawn to that section of the symposium report, concerning social, ethical, legal and regulatory aspects, which deals with these matters most fully.

Foremost in my mind during these discussions was our difficulty in assessing the urgency of the demographic crisis. To the extent that the impact of that crisis increases, the need for more effective family planning technologies must increase. At the very least, failure to develop something that may provide a more effective technology would be to take a grave and unnecessary risk. This is a personal view, and I am fully aware of the threat posed by the term 'effective instrument'; but it seems to me true and important.

We can look forward to a time in the not too distant future when the effort needed to search for new antifertility vaccine molecules will no longer be a limiting factor. By then we shall have plenty of candidate molecules to work with. Evaluation and safety testing will still be laborious and important, but here also we can expect molecular biology to make valuable contributions. For instance, by placing human genes in transgenic mice, we may be able to gain the benefits of small animal experimentation while at the same time being able to examine directly the human proteins that are our ultimate targets. What is much more likely is that the limiting factors will lie in the sociology of these future vaccines. It will be their acceptability, safety, cost and ability to fulfil human needs that will decide how rapidly they can be introduced into general use, and how wide the scope of their application will be. That is why the message from this symposium to researchers and to governments is so important.

16 Report of the symposium: Points to consider in the assessment of the safety and efficacy of vaccines to regulate fertility

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Introduction

As indicated in the Preface to this volume, five principal topics were identified for detailed discussion in the symposium.

(1) The selection of antigens for antifertility vaccine development.
(2) Preclinical safety and efficacy studies with antifertility vaccines.
(3) Clinical trials of antifertility vaccines.
(4) Long-term surveillance of antifertility vaccine use.
(5) Social, ethical, legal and regulatory aspects of antifertility vaccines.

This report summarizes the views and recommendations of the symposium participants concerning the field in general and each of the above five topics in particular. It is intended as a forward-looking document to be revised as new information becomes available. It should not be regarded, therefore, as a comprehensive and rigid set of guidelines but rather as a source of points to be considered when deciding on the direction and objectives of future research in this rapidly evolving area.

The selection of antigens for antifertility vaccine development

There is a large number of molecules associated with, and necessary for, virtually all of the many and complex biological events of human reproduction. Although fertility can be impaired, both naturally and experimentally, by eliciting an immune response to many of these molecules, this is often accompanied by side-effects which would make some of them unattractive candidates for antifertility vaccine development. It is necessary, therefore, to establish criteria for the identification and selection of suitable candidates and to determine the feasibility of developing and evaluating antifertility vaccines based on these molecules.

Criteria for the selection of candidate immunogens

In identifying and selecting candidate molecules to be used as immunogens in the development of antifertility vaccines, criteria relevant to safety, efficacy, acceptability and feasibility need to be used.

Safety criteria

(a) The molecules need to be reproduction specific, i.e. their expression should be confined to the gametes, certain reproductive tissues, certain reproductive hormones, and/or other products of the early conceptus.
(b) The molecules should not be produced by, or expressed on, normal body tissues other than the intended vaccine target and should be restricted to a site where a controlled immune response will not produce adverse side-effects.
(c) Immunization with these molecules should be free of significant acute or long-term side-effects, including unwanted autoimmune, allergic or toxic reactions.

Efficacy criteria

(a) Active immunization with these molecules must disrupt fertility.
(b) Preferably, the molecules should be present transiently and in relatively low amounts so as not to overwhelm the predicted immune (antibody) response.

Acceptability criteria

(a) To be acceptable as a family planning method, antifertility vaccines should not function as abortifacients but should exert their effects before the process of implantation has been completed.
(b) The mechanism by which immunization against the molecule achieves an antifertility effect should be known or suspected.
(c) The antifertility effect produced by immunization with these molecules should not be permanent, and fertility should return after vaccine-induced immunity has waned.
(d) The vaccine should be pharmacologically inert, according to the standard criteria laid down by regulatory authorities, and should not produce any unacceptable metabolic, endocrine or other disturbances.

Feasibility criteria

(a) The immunogen should be amenable to large-scale production by a cheap and reliable process and the product should be capable of satisfying accepted quality control procedures. This requires that the precise chemical structure of the molecule is known or can be readily deduced.
(b) The vaccine should be suitable for widespread distribution and use in both developing and developed countries.
(c) Relevant animal models must be available for preclinical studies of

vaccine safety and efficacy. This requirement is equally applicable to all of the above criteria categories.

Antifertility vaccine candidates

The function of many of the molecules involved in, and specific for, the process of reproduction can be inhibited immunologically to bring about an antifertility effect. In many cases, however, this desired effect is accompanied by undesirable side-effects, such as endocrine and other metabolic disturbances, which would make many of these molecules unacceptable candidates for vaccines intended for use in humans. Furthermore, the unknown consequences of chronic immunity to 'self' molecules in the brain, pituitary gland and gonads would argue against using immunogens restricted to these sites in the development of vaccines to be used for family planning purposes.

On the basis of the criteria referred to earlier, the most promising candidates are antigens which are found in the membrane of ejaculated sperm, stage-specific antigens in the zona pellucida surrounding the ovum (preferably post-ovulation) or antigens in the trophectoderm of the pre-implantation conceptus. These molecules may be restricted to the lumen of the reproductive tract (e.g. non-secreted components of sperm, ova and the pre-implantation conceptus) or appear in the circulation (e.g. secreted products of the conceptus, such as hCG).

In the case of vaccine targets in the circulation, systemic immunity may be sufficient to produce an antifertility effect, either by bioneutralization or enhanced clearance of the target molecule as a result of the formation of antigen–antibody complexes and their uptake by the reticuloendothelial system.

In the case of target molecules restricted to the lumen of the male or female reproductive tracts, systemic immunity in the form of antibody and/or sensitized cells could be effective if transported in sufficient quantities from the circulation to the lumen of the reproductive tracts. However, it is likely that mucosal immunity may play a more significant role in these locations.

Mucosal immunity

Cells producing secretory immunoglobulin A (sIgA) are present in the epithelium of the oviducts, cervix and vaginal wall but appear to be lacking in the endometrium, seminiferous tubules, vas deferens and epididymis. By contrast, the latter sites contain many macrophages, large granulated lymphocytes and T cells.

The potential therefore exists to stimulate a mucosal sIgA response in the female reproductive tract to sperm, ova and/or the pre-implantation conceptus. Similarly, it might be possible to elicit a cell-mediated mucosal immune response in the male reproductive tract to integral or coating components of the sperm plasma membrane.

For sperm-based vaccines to be effective, in either the male or the female, a local mucosal response is desirable since there is no guarantee that systemic antibodies will exert a protective effect by transudation into the reproductive tract.

On the other hand, sIgA production is T lymphocyte dependent and a sustained response requires induction of immunological memory in T cells as well as B cells. Thus mucosal stimulation with self antigen alone is unlikely to be effective. Whilst immunization via the genital tract may be the route through which antisperm immunity is elicited in some infertile individuals, the experimental induction of local immunity by this route does not appear to be possible without an accompanying systemic response. Recent work in primates does, however, indicate that good sIgA responses can be obtained in the female reproductive tract by initial systemic immunization followed by an oral boost. This approach is feasible in humans and might eventually result in the use of either microcapsules or live vectors for presentation of the vaccine candidate.

As the development of antisperm vaccines is considered a high priority, there is a need for future research to focus on developing the necessary reagents and animal models to test the feasibility of inducing a sustained sIgA response to selected sperm antigens in both the female and male reproductive tracts. Although evidence is lacking that relevant regions of the human male reproductive tract can be induced to be immunologically active against sperm, in a way that will impair fertility without inducing autoimmune reactions and pathology, the feasibility of developing a vaccine which would exert its effect by stimulating a local sIgA or cell-mediated immune response in an appropriate animal model should be examined.

Valuable information regarding the production of mucosal immunity might be obtained from ongoing clinical trials involving mucosal immunization against infectious agents. In particular, data on the correlation of salivary with vaginal sIgA levels and the duration of vector-induced antibody responses could become available by carrying out collaborative studies with investigators engaged in this area.

Another major advantage of mucosal immunity is the avoidance of the theoretical risk of systemic IgG class antibodies crossing the placenta and affecting the early fetus in the event of pregnancy occurring in an immunized woman.

Evaluation of the efficacy of candidate immunogens

Once potential vaccine candidates have been identified and prepared, by biochemical, immunochemical and/or molecular genetics techniques, they need to be subjected to rigorous in-vitro and in-vivo tests to determine whether they meet the efficacy criteria. Although the procedures will differ according to the immunogen being used and the known or suspected mechanism of action

of the prototype vaccine, the following general outline is applicable to all cases.

To date, all potential immunogens have been selected on the basis of their capacity to elicit a specific antibody response. These tests, therefore, refer to the evaluation of antibodies rather than cell-mediated immunity (CMI), although tests for CMI may need to be included in the future.

The testing of antibodies elicited by prototype vaccines

In-vitro tests should include biochemical and biological assay systems, for example:

(a) simple, rapid binding assays (radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), etc.);
(b) assays of binding capacity and affinity;
(c) reactions with target and non-target tissues in the human and species cross-reactivity (more relevant to safety than to efficacy);
(d) receptor binding/blocking (where relevant);
(e) enzymatic analysis (where relevant);
(f) bioassays (e.g. sperm immobilization, cytotoxicity, etc.).

If the in-vitro assays confirm that the antigen elicits a potentially useful antibody response, further studies should be carried out *in vivo*.

In-vivo studies should include:

(a) neutralization of biological activity of the target antigen in relevant animal models;
(b) antigen challenge tests in actively immunized animals;
(c) determination of antifertility effects.

Depending on the interspecies distribution of the antigen target, it may be possible to carry out initial efficacy experiments in a readily available small animal (rat, rabbit, etc.). However, efficacy studies in an infrahuman primate are usually required by regulatory authorities.

Preliminary vaccine development, composition and delivery

In the light of past experience, current technology and prospects for the immediate future, candidate immunogens are likely to be derived from one of two classes of chemical compounds, polypeptides and carbohydrates. Each has its advantages and disadvantages in terms of vaccine development.

Polypeptides as antifertility immunogens

The major advantage of polypeptide immunogens is that they can be readily produced either by classical chemical synthesis methods or by biosynthetic procedures using recombinant DNA technology.

Peptides have attracted a great deal of interest in the development of

synthetic vaccines for a variety of intended applications. They can be designed and synthesized so that they express single or multiple epitopes of the required specificity and can be manufactured in bulk with adherence to strict quality control requirements. In general, peptides of less than 50 amino acids in length are prepared by chemical synthesis, while longer peptides are produced by recombinant DNA techniques.

A potential disadvantage of peptide immunogens is that single epitope immunogens may not be universally effective. This is particularly true of T-cell epitopes where genotype differences (genetic restrictions) will affect immune responses. The use of multiple epitope peptides should overcome this problem to a large extent.

Carbohydrates as antifertility immunogens

A major attraction of carbohydrate immunogens is that these molecules, in the form of glycosylated proteins, appear to be involved in gamete interactions during the process of fertilization.

Although carbohydrates can be synthesized enzymatically or chemically, this is still a difficult and costly exercise. However, recombinant DNA techniques are now available for the production of glycosylated protein antigens.

The principal disadvantage of pure carbohydrates as antifertility immunogens is that they do not produce memory cells. The immune response consists predominantly of short-lived immunoglobulin M (IgM) – with only some IgG2 and IgG4. This may not be a problem if the immunogen persists and intravascular neutralization alone is sufficient for efficacy to be achieved. Another major disadvantage of carbohydrate immunogens is their lack of tissue-specificity.

Vaccine design

Broadly speaking, vaccines can take two forms. They are either composed of a purified or synthesized antigen conjugated to a large molecular weight carrier and given with an adjuvant (conventional vaccines), or they can consist of a recombinant DNA vector containing the gene for the defined antigen, with or without a carrier molecule gene, which is expressed in a prokaryote or eukaryote system (vector-based vaccines).

Conventional vaccines

Examples of conventional vaccines are the human chorionic gonadotrophin (hCG) vaccines which have been evaluated recently in Phase 1 clinical trials. In these vaccines, either the complete beta subunit of the hormone, or a synthetic peptide representing the carboxyterminal region of the beta subunit (β-hCG–CTP), is conjugated to a toxoid carrier molecule, mixed with alum or muramyl dipeptide (MDP) to act as an adjuvant and administered in a fluid vehicle.

Carrier molecules are required to increase the immunogenicity, particularly

of peptides, by stimulating the T cell compartment of the immune system to provide help for antibody production. To date, most of the work with hCG vaccines, for example, has relied on the use of tetanus and diphtheria toxoids as carriers. In general, these vaccines have proved to be immunogenic and relatively free of side-effects, although the antibody response generated to hCG has been somewhat variable, and it has also been necessary to use an adjuvant.

Alternative carriers have been evaluated (e.g. purified protein derivative (PPD), recombinant mycobacterial antigens) but none appears superior to the toxoids. Similarly, alternative adjuvants to alum and MDP have been tested. The problem with using more potent adjuvants is that unwanted side-effects (local and autoimmune) might increase to unacceptable levels. To overcome these problems, the following modifications could be made to the way in which the immunogen is presented to the immune system of the vaccine recipient.

(a) *Incorporation of the immunogen into a slow-release delivery system.* To this end, iscoms, liposomes and co-polymer microspheres have been tried, of which the last-mentioned have proved the most promising for the β-hCG–CTP vaccine. The additional advantage of this approach is that it should reduce, perhaps to only one, the number of injections required to obtain a sustained and effective antifertility immune response. In addition, adjustments can be made in the formulation of the microspheres to alter the duration and rate of release of the incorporated immunogen. Furthermore, these preparations may be given orally to generate an sIgA response at mucosal surfaces. A major advantage of some of these copolymers is that a substantial amount of toxicology has been carried out on them and at least one has been approved for clinical use.

(b) *Administration of the conjugate, or even a defined peptide alone, in association with heterocyclic stimulants or interleukins.* At present, the use of this approach is under active scrutiny by industry as an alternative to the use of adjuvants, although it is unclear whether such an approach will reduce the number of injections required or be practical in developing countries for logistic and financial reasons.

Vector-based vaccines

Expression of the genes for defined protein and peptide antigens, and more recently carbohydrate antigens, in a suitable microbial vector is now a realistic option for vaccine development programmes. The vector can substitute both for the carrier and adjuvant and, depending on which vector is selected, both systemic immunization (e.g. with vaccinia) or via mucosal surfaces (e.g. with salmonella or adenovirus) may be possible.

The following three issues are of special concern in connection with the use of these vectors for delivering antifertility vaccines.

(a) The safety of the vectors, particularly when used over extended periods of time.
(b) The duration of immunity and the possibilities for reversing the antifertility effect.
(c) The possibility of vector transmission between individuals.

Much research is ongoing in this area, particularly in connection with the use of live vectors in the development of anti-infectious disease vaccines. The information to be generated in these studies will be of major importance in determining if live vectors can be considered for use in antifertility vaccines.

Antifertility vaccines and HIV infection

Over 140 countries have reported cases of acquired immune deficiency syndrome (AIDS) and the prevalence of human immunodeficiency virus (HIV) infection is believed to be increasing in most of these countries. Since HIV can lead to both immunodeficiency and autoimmune disease, great care is required when administering vaccines or other foreign antigens, particularly on a repeated basis. With respect to killed vaccines against infectious disease, it is generally accepted that the benefits from vaccination outweigh any potential disadvantages. The situation is quite different, however, for antifertility vaccines which can involve the administration of self or self-like antigens, which depend on an intact immune system to be effective, and which are given to fertile adults rather than children. The following recommendations are made, therefore, in connection with this issue.

(a) That evidence of HIV infection be an exclusion criterion for Phase 1, 2 and 3 clinical trials of antifertility vaccines.
(b) That research be carried out, as a matter of priority, on possible interactions between HIV infection and antifertility vaccines to identify any potential effects of the vaccines on the course of HIV infection and any potential effects of HIV infection on the efficacy and safety of the vaccines.
(c) That antifertility vaccines should not be considered among the preferred options for birth control in populations with a high prevalence of HIV infection, until concerns about the safety and efficacy of these vaccines in immunocompromised individuals have been resolved.

Preclinical safety and efficacy studies with antifertility vaccines

Because of their novelty, there are few, if any, national or international requirements for the preclinical safety and efficacy testing of antifertility vaccines, and the approvals that have been obtained to date for conducting

clinical trials with such vaccines have resulted largely from *ad hoc* requests and discussions with national regulatory authorities. In view of the rapid developments that are occurring in the vaccine field, it is difficult to predict the compositions and mechanisms of action of future antifertility vaccines, and this, in turn, makes it difficult to define, with any precision, the nature and duration of preclinical studies to be carried out with these preparations. The remainder of this section of the report, therefore, addresses general issues that need to be taken into account when deciding upon such studies rather than details of the studies themselves.

Issues to be considered

As antifertility vaccines will be used to inhibit a physiological function, rather than to combat external pathogens, they could be considered to be both vaccines and drugs. They also have the unique property of being based on immunogens which have been constructed and presented to the immune system to induce a specific response against a particular host molecule. There are, therefore, special considerations in the preclinical safety and efficacy studies of antifertility vaccines that apply neither to conventional vaccines nor to drugs.

By virtue of their mechanism of action, special attention needs to be paid in preclinical studies to the possibility of adverse immunological side-effects resulting from the use of antifertility vaccines. It is always difficult to extrapolate from preclinical animal studies to the eventual human situation, and it is particularly difficult when an assessment is being made of the immunological side-effects of immunization of animals with prototype vaccines based on human immunogens. For these reasons, great care needs to be taken in making recommendations that are intended to guide future preclinical and clinical studies in this area.

In determining and designing the nature and range of the preclinical studies to be carried out with novel antifertility vaccines, a number of factors need to be considered. These include the need for, and feasibility of, carrying out routine and specialized toxicity, immunosafety, teratology, carcinogenicity and mutagenicity studies with these preparations, as well as the identification and selection of appropriate animal species in which these studies should be conducted. Fundamental to these issues and to the design and intepretation of these studies is the composition of the vaccine and its known or suspected mechanism of action.

Composition of the vaccine

It is essential that the vaccine used in the definitive preclinical studies is the same formulation (and preferably the same batch) that will be used in the subsequent clinical trial. Accredited documentation giving details of the

chemical composition, method of manufacture and source of supply of all the components of the vaccine, together with the appropriate quality assurance certification, will be required.

From experience gained with the development of anti-hCG vaccines, the dosage form of the first generation of antifertility vaccines is likely to consist of the following components.

(a) The target-specific component of the immunogen derived from, or based on, a molecule involved in the reproduction process: this will most likely be a synthetic peptide or a recombinant DNA product consisting of either protein alone or a protein–carbohydrate (glycoprotein) molecule. Full documentation on the chemical composition of the immunogen, its method of preparation and quality assurance testing need to be provided.

(b) The carrier component of the immunogen: this may be a novel synthetic carrier but is more likely to be an established product like tetanus toxoid or diphtheria toxoid which will already have well established and documented production and quality assurance procedures.

(c) Conjugation procedure: the composition of the coupling reagent and the chemistry of the coupling procedure, as well as the relative proportions of coupling reagent, immunogen and carrier in a human dose of the conjugate need to be described.

(d) The adjuvant (immunostimulant) component of the vaccine: the chemical composition of the adjuvant and details of its manufacture and quality assurance need to be documented. More detailed information will be needed for any novel chemicals not previously used in humans.

(e) The vaccine vehicle or delivery system: this component of the vaccine could comprise a water-in-oil emulsion or a biodegradable/biocompatible copolymer microsphere together with a suspending medium. In each case, the chemical structures, relative proportions, manufacturing and quality assurance procedures for all components need to be documented.

A consistent method of production of the final vaccine should be established, under conditions of Good Manufacturing Practice or an equivalent standard. Data on the sterility, pyrogenicity and stability of the vaccine should be established before embarking on extensive preclinical safety and efficacy studies.

The preclinical safety testing of antifertility vaccines

An evaluation of the general safety of antifertility vaccines will be derived from routine animal toxicology and clinical pharmacology studies. However, the

composition and mechanism of action of these novel preparations dictate that immunological and endocrinological studies play an important role in the overall strategy of toxicity testing. These studies should include tests in both animals and humans, especially at the Phase 1 and Phase 2 clinical trial stages. Essential to a meaningful evaluation is the identification and use of relevant animal models and appropriate design of the immunological and endocrinological studies.

Selection of animal models

The immunogens selected for antifertility vaccine development will be tissue specific and may be primate-specific or even species specific. This may preclude the use of rodents and other non-primate species in the immunotoxicological tests as such animals may be poor predictors of immunotoxicity in the human. The following points should be considered, therefore, when designing these studies and selecting the animal models to be used.

(a) When the antigen on which the vaccine is based is not represented in the animal species to be used for routine immunotoxicity tests, an additional animal species should be sought in which an antigen of similar structure, function and location to the human counterpart is present. Furthermore, it is desirable that the immune response elicited in the animal model by the heterologous human immunogen will cross-react with the endogenous animal antigen and that the nature and sequelae of the immune response elicited in the animal are similar to those to be expected in the human situation.

However, it is recognized that it may not be possible to achieve this ideal situation since molecules with the same structure and function are rarely conserved across species, even in those closest to humans. Under these circumstances, the most relevant animal model should be chosen, paying particular attention to similarities in the structural, functional, immunogenic and immunoreactive properties of the vaccine target in the animal compared to the expected clinical situation.

(b) Ideally, an homologous vaccine, using the animal model antigen as the immunogen, should be developed and evaluated in parallel with the prototype vaccine incorporating the human immunogen.

(c) In addition to identifying the appropriate animal species, it would be advisable to use animals of the same sex in which the vaccine is intended to be used in the human.

Immunological studies

In addition to conducting safety studies at the immunization schedule proposed for human use, a hyperimmunization study should also be carried out in an attempt deliberately to produce adverse events which may occur only

at low frequency or after extended periods of vaccine use in humans. The immunization schedule should be designed to maximize the production of any adverse events, although daily administration of vaccine, as often used for standard toxicology testing, should be avoided since it may induce immune tolerance rather than high levels of immunity. The overall observation period and the timing of the examinations should be designed to optimize the chances of detecting adverse effects. The types of test to be carried out are indicated below.

Immunological tests to be carried out in animal studies

The following tests relevant to an assessment of the immunosafety of the vaccine should be carried out at the preclinical stage.

(a) Standard histopathology.
(b) Tests for reaginic antibodies.
(c) Tests for the presence of potentially pathogenic immune complexes in serum, skin, kidney and other appropriate tissues.
(d) Tests for delayed hypersensitivity.
(e) Tests of antibody specificity, using procedures such as ELISA, radioimmunoassay and immunofluorescence and immunoperoxidase techniques to determine if the antibodies generated to the immunogen cross-react with non-target constituents of body fluids and tissues.

Endocrinological studies

When the immune response elicited by the immunogen is directed against a hormone or a growth factor, endocrine or metabolic imbalances and disturbances may be produced. Therefore, animal experiments, clinical trials and follow-up studies should include appropriate laboratory and functional tests to evaluate relevant endocrine systems.

Toxicological studies

Protocols for animal toxicity studies should take into consideration the intended clinical use of the vaccine, particularly the dose, frequency, and mode and route of administration.

All components of the final formulation, including the specific immunogen, the carrier molecule, immunostimulant, delivery system and vehicle should be tested for toxicity. Separate toxicity tests should be carried out on these components individually, as selected combinations and as the complete formulation. Only selected novel combinations and/or the complete formulation may need to be tested if toxicological information is already available for any of the components from previous studies in either animals or humans.

Acute and subacute toxicity studies

Acute, single administration toxicity studies should be conducted in two species. The doses used should be the equivalent of one and ten times the human dose on a weight for weight basis. There is no scientific rationale for conducting LD50 studies with an antifertility vaccine, and such studies should not be performed.

For repeated-dose, subacute studies, the frequency of administration of the vaccine should take into account the intended use in the human and the nature and time-course of the immune response elicited by the vaccine.

Chronic toxicity studies

Conventional long-term toxicity studies are unlikely to be required for the complete vaccine formulation since relevant safety data will have been obtained from the immunosafety tests and particularly from hyperimmunization studies.

Reproduction and teratological studies

Reproduction and teratological studies should be carried out in the most appropriate animal species depending on the type of immunogen and its known or suspected mechanism of action. Guidelines for these studies are well established and include effects on gonadal function, estrous and menstrual cyclicity, mating behaviour, conception rate, early stages of pregnancy, embryotoxicity, teratogenicity, fetal development, lactation, neonatal viability and growth of the newborn.

Teratology studies should investigate both the chemical teratogenicity of the vaccine and its breakdown products, as well as the immunoteratogenicity of the immune response elicited by the vaccine. These studies should be carried out under various conditions of immunization, e.g. during the periods of waxing and waning immunity as well as at the times of maximal effect.

Evaluation of potential hazards to the fetus and infant

Fetuses obtained from immunized animals that become pregnant in the presence of ineffective immunity should be examined both macroscopically and microscopically for malformations and endocrine abnormalities. Particular attention will be directed to organs considered to be at high risk of potential damage because of the type of immunogen used and its known or suspected mechanism of action. It may be valuable to allow some pregnancies to continue to term and to study the progeny through to sexual maturity.

Carcinogenicity and mutagenicity studies

The predictive value of the routine tests for mutagenicity and cytogenetic aberrations requested by some regulatory authorities is far from clear in

relation to antifertility vaccines. It is unlikely that carrying out such tests with individual components, selected combinations or the complete vaccine formulation would yield meaningful data.

The need to carry out long-term carcinogenicity studies will depend on the type of immunogen used and the known or suspected mechanism of action of the vaccine. For example, vaccines that inhibit the biological functions of a hormone may need to be considered for carcinogenicity studies if there are reasons to suspect there is a danger of tumour initiation and/or promotion in susceptible tissues as a direct or indirect result of changes in the levels or biological activity of the hormone.

The preclinical efficacy testing of antifertility vaccines

Efficacy is usually demonstrated before carrying out major preclinical toxicity and immunosafety studies and must be established before proceeding to clinical trials. Although it may be possible to carry out laboratory tests *in vitro* in order to provide an index of potential efficacy, definitive in-vivo efficacy studies in relevant animal models must also be conducted.

In-vitro tests of efficacy

Depending on the immunogen used in the vaccine and its intended mechanism of action, a series of in-vitro tests can be performed that may indicate potential efficacy.

(a) *Binding tests.* The ability of the elicited antibody to bind to the target molecule can be assessed, both qualitatively in terms of target specificity, and quantitatively in terms of binding affinity.

(b) *Tests for inhibition of target molecule structure and function.* The ability of the elicited antibody to inhibit the target molecule can be assessed. For example, antibodies to hCG can be assessed for their ability to inhibit growth of the trophectoderm and antibodies to sperm can be assessed for their ability to agglutinate and/or immobilize sperm.

Similarly, the ability of the elicited antibody to inhibit biological functions can be demonstrated *in vitro*. For example, antibodies to hCG can be assessed for their ability to inhibit hCG-stimulated steroid production by gonadal cells in culture, and antibodies to sperm and/or ovum can be assessed for their ability to prevent gamete interactions and fertilization *in vitro*.

In-vivo tests of efficacy

An indirect index of potential efficacy can be obtained by assessing the ability of antibodies elicted by the vaccine to inhibit biological function *in vivo*. For example, antibodies to hCG can be assessed for their ability to inhibit hCG-

stimulated uterine weight gain when passively administered to sexually immature ovariectomized rodents. In addition, antisperm antibodies can be assessed for their ability to prevent fertility in experimental animals when sperm, exposed to the antibodies *in vitro*, are administered by artificial insemination to isologous females.

A direct test of the in-vivo efficacy of the candidate vaccine should also be carried out in relevant animal models, usually including at least one infrahuman primate species. Ideally, these in-vivo studies will involve comparative evaluations of both the heterologous (human) immunogen and the homologous (animal) counterpart.

Clinical trials of antifertility vaccines

The major objectives of clinical trials of new drugs are:

(a) to demonstrate a pharmacological action in humans which is likely to be useful in the treatment of the condition for which the drug has been developed;
(b) to assess the dose–response characteristics with special emphasis on interindividual variability and margin of safety;
(c) to devise dosage schedules to be used in the continued clinical evaluation of the drug;
(d) to devise and validate methods for monitoring subjective and objective drug responses in the later phases of drug evaluation.

The clinical testing of antifertility vaccines, however, is a new area in which there is little relevant previous information and experience to guide the investigator. In determining the studies required, as well as in designing the protocols for such studies, it is necessary to draw upon the experience gained in the clinical testing of antidisease vaccines, steroidal contraceptives and other drugs.

Phases of testing

Conventionally, the clinical testing of drugs destined for human use is divided into the following three principal phases, each of which has specific objectives, as indicated.

Phase 1 A: Clinical pharmacology (pharmacodynamics and pharmacokinetics) of the drug in healthy volunteers.
B: Preliminary dose-finding studies in healthy volunteers.
Phase 2 A: Clinical pharmacology of the drug in patients.
B: Definitive dose-finding studies in patients.
Phase 3 Extended clinical trials in patients for clinical efficacy, acceptability, and assessment of the nature, extent and severity of side-effects.

These conventional definitions need to be modified in the case of clinical trials with antifertility vaccines, and the specific requirements and design of clinical trials with vaccines directed at different reproduction-specific antigens may vary and will be influenced by the known or suspected site and mechanism of action of each vaccine.

The general objectives, overall design and specific requirements of Phase 1, 2 and 3 clinical trials of antifertility vaccines are described below and summarized in Table 16.1. This information is drawn primarily from the experience gained with hCG vaccines as these are the only antifertility vaccines, to date, to have been evaluated clinically.

Phase 1 clinical trial

Purpose

The primary objective of a Phase 1 clinical trial of a new antifertility vaccine is to determine the safety, but not the efficacy, of the preparation in humans. However, an indication of potential efficacy can be obtained at the Phase 1 trial stage by assessing the nature, magnitude and duration of the immune response and relating this to the level of immunity estimated to confer efficacy in fertile individuals.

Selection of trial centres

In view of the exploratory nature of a Phase 1 clinical trial of a novel antifertility vaccine, a large number of physical examinations and endocrinological and immunological laboratory tests need to be carried out with the minimum of delay in order to detect adverse side-effects at the earliest possible stage. Phase 1 trials of these vaccines, therefore, are (ideally) carried out in a single centre at which all of the required facilities are available.

Subject numbers

In order not to expose any more subjects than necessary to unknown risks, the number of subjects recruited to a Phase 1 clinical trial should be the minimum required to obtain reliable data. The lower limit is set by the number of subjects needed to establish a dose–response relationship with at least five dose levels of the vaccine – with five to six subjects per dose group as controls – whereas the upper limit is set by logistic considerations. Generally, about 50 subjects are required.

Selection of subjects

Phase 1 trials of new methods of family planning require volunteers who are not at risk of pregnancy, in that safety, immunogenicity and dose–responsiveness, but not efficacy, is to be assessed. Optimally, they should be of reproductive age, no younger than 25 years, and have had children previously. These criteria are necessitated by the unproven reversibility of the method. These optimal criteria are difficult to attain in most study populations. Phase 1

Table 16.1. *Requirements for the conduct of clinical trials of fertility-regulating vaccines*

	Phase 1	Phase 2	Phase 3
Definition	Safety and immunogenicity Dose-response characteristics	Preliminary efficacy Further safety assessments Complementary studies where at least one booster immunization will be given	Extended efficacy Safety and acceptability Further assessment of booster immunization
Number of subjects involved	Up to 100 Generally around 50	Around 750 cycles Subject numbers depend on predicted duration of effective immunity	At least 10 000 cycles of exposure Control groups if possible
Pre-trial requirements	Standard animal toxicology Immunotoxicology in an appropriate animal model To include hyperimmunization studies	Standard animal reproductive toxicology (including teratogenicity) Immunoteratology studies in an appropriate animal model to be at least initiated or preferably completed	Standard animal carcinogenicity studies
Drug regulatory requirements	Conform with national agency regulations for country of proposed trial and (where applicable) for country of export of vaccine components		
Ethical approval	In accordance with Declaration of Helsinki (1975) and guidelines laid down by Council for International Organizations of Medical Sciences (1982). Approval by Ethical Review in sponsoring organization and by an appropriate Ethics Committee of the Trial Centre/Institution		
Legal indemnity	Provision must be made for compensation of trial subjects experiencing trial-related injury by legal indemnity of (1) responsible investigator; (2) trial centre/institution; (3) trial sponsor		
Selection of trial centres	Availability of comprehensive specialized clinical and laboratory facilities	Availability of facilities for acute monitoring of onset and offset of potential efficacy and high-level laboratory clinical, and family planning facilities May involve selected centres in less-developed countries to allow study of different population characteristics	Multiple centres in developed and less-developed countries Common protocols

Table 16.1. (*cont.*)

	Phase 1	Phase 2	Phase 3
Recruitment of subjects	Strategy will vary with nature of vaccine and trial phase. Avoid misleading publicity concerning the status of the vaccine and nature of the trial. Financial re-imbursement in accord with WHO Guidelines: reasonable expenses for travel, child-minding, time off work, etc; level of re-imbursement should not constitute an inducement to participation		
Informed consent	As for 'ethical approval'. Provision of comprehensive written information on background, risks, benefits, follow-up and commercial implications prior to obtaining written consent		
Subject and selection inclusion criteria	Sterilized or reliably contracepting Reproductive age and older than 24 years Will have had children previously Healthy Menstruating normally	Healthy Reproductive age (e.g. 18–35 years for females; 18–50 years for males) Achieved at least one previous pregnancy Stable, potentially fertile relationship Normal ovulatory menstrual cycle	Individuals requiring contraception
Exclusion criteria	Pregnant or breast-feeding High risk or presence of significant systematic disease, allergies or drug reactions Significantly abnormal laboratory and physical screening tests Drug addiction Identified risk of reaction to vaccine component HIV antibody or hepatitis B sAg positivity	As for Phase 1	Pregnancy Immunosuppressive disease Malignant disease Severe debilitating illness
Screening/entry assessment	History and physical examination Cervical smear ECG Ophthalmoscopy Urinary tests Haematology Biochemistry Hormone assays	Modified from Phase 1	Routine background laboratory, clinical and sociodemographic data

	Immune serology Immune response assays for antibodies to vaccine antigen or other relevant antigens		
In-trial monitoring	*Safety* Interval assessment of selected entry parameters Acute phase assessment following immunization Adverse reaction recording Menstrual diary *Immune response* Antibody binding and bioactivity assays at appropriate intervals	*Efficacy* Occurrence of pregnancy Rapid screening test or procedure to identify potentially effective immunity *Safety* Adverse reaction recording Interval assessment of selected entry parameters Menstrual diary	Pregnancy Adverse effects Rapid screening for protection Interval assessment of selected parameters identified in Phases 1 and 2
Exit assessment	As for entry	As for entry	As for entry
Discontinuation criteria	Subject: Wishes to withdraw Develops significant study-associated condition Trial: Significant proportion of subjects develop clinically unacceptable trial-related disorders	Subject: Wishes to withdraw Fails to respond adequately Develops significant study-associated condition Pregnancy Trial: 2 or more subjects become pregnant Unacceptable trial-related disorders	Subject: As for Phase 2 Trial: Unacceptable pregnancy rate
Follow-up	As indicated by persisting evidence of immune response or adverse effects	As indicated Of offspring of pregnancies occurring during trial and in subsequent 3 years	As for Phase 2
	Note: Case record and administrative procedures should be established in all trials to facilitate long-term follow-up of subjects and any offspring		
Research and ancillary studies	All research studies/tissue sampling etc. which lie outside the goal-oriented objectives and requirements of the trial should be the subject of a separate formal research protocol		

subjects will usually be required to have been previously electively sterilized or reliably contracepting using a method, such as the intrauterine device, which does not interfere with either the activity of the vaccine or with the assessment of laboratory and clinical parameters. Hormonal contraceptive users are unacceptable because of the possibility that these methods might influence immune responses and, in the female, will mask menstrual irregularities, will not allow monitoring of ovulation, and will disturb hormone and biochemical indices.

Recruitment

To facilitate recruitment, it may be necessary to disseminate information about the Phase 1 trial using limited and carefully controlled publicity. There should be no unethical advertizing, e.g. misrepresenting the benefits of the preparation or treatment or the nature of the trial. No coercion or financial inducements should be made or implied although reimbursement of the travel costs and reasonable compensation for lost earnings and other legitimate expenses resulting from participation in the study may be made on an individual basis.

Informed consent

One of the most important aspects of a clinical trial is ensuring that the subject's consent to participate in the study is given freely and only after all relevant information has been provided in a detailed and fully understandable manner and to the complete satisfaction of the subject. In order to give informed consent to participate in a clinical trial of an antifertility vaccine, subjects must understand the following points.

(a) The experimental nature of the vaccine.
(b) The goals of the study.
(c) The nature of the antigen used, including its role in reproduction.
(d) The likely mechanism of action of the vaccine.
(e) The results of prior studies of the vaccine, including adverse reactions.
(f) Theoretical risks including:
    adverse reactions not seen in previous studies;
    irreversibility of antifertility effect;
    failure of antifertility effect resulting in unplanned pregnancy (in Phase 2 and 3 trials).
(g) Options for dealing with unplanned pregnancy should it occur, including termination if legal, and carrying the pregnancy to term with the possible risk of adverse pregnancy outcome.
(h) Compensation available for subjects who experience adverse reactions. This information may include the source of compensation and the spectrum of coverage (e.g. medical treatment, lost income, pain and suffering).

(i) Alternative methods of contraception available.
(j) Likely advantages of this form of contraception.
(k) Real and theoretical benefits from participation in the study.
(l) Reimbursement for lost earnings and legitimate expenses.
(m) Study design including:
eligibility criteria for participation;
number of participants and centres;
duration of study;
number of vaccine doses and route of administration;
number and nature of visits: in particular, procedures potentially considered invasive should be outlined (e.g. blood drawing, HIV testing, physical examinations, injections, skin tests).
(n) The need to be recontacted in the course of follow-up studies.
(o) Provisions for confidentiality of patient records. (In general, patient information is restricted to study personnel, within the limits of the law. Data are published in such a way that identity of individual patients is not possible.)
(p) The name and telephone number of a person who may be contacted for further information pertaining to the trial or for reporting of adverse reactions.

Exclusion criteria

As a Phase 1 clinical trial is the first time that a novel preparation is used in humans, the exclusion criteria are, of necessity, extensive and no individuals should be recruited who are considered to be unsuitable or who might reasonably be expected to be subjected to an unacceptable health risk by taking part in the study.

Assessment of immunogenicity

The immunogenicity and duration of action of an antifertility vaccine are assessed by the serial measurement of antigen-binding and antigen-neutralizing capacity, in serum. Theoretical calculations can be made to give an indication of the serum concentrations of antibody that would be necessary to neutralize physiological amounts of the target antigen, taking in account its location, i.e. in the circulation or in the lumen of the reproductive tracts.

Additional information relevant to the utility of the vaccine may be gained by antibody isotype analysis, and by in-vitro assays of T lymphocyte recognition and response. If the latter reveal the presence of immunological memory to the vaccine antigen, there may be a greater chance of stimulation whenever the self antigen reappears and hence a less likely chance of reversibility of the antifertility effect.

Immune reactions to the carrier (e.g. diphtheria toxoid) may also be studied for evidence of both cell- and antibody-mediated responses. Such data may assume importance if repeated booster doses of the same vaccine are

envisaged, and are also relevant to the potential prophylactic effects of immunity developing to other carriers such as tetanus toxoid.

Assessment of safety

The laboratory assessments of the safety of antifertility vaccines should pay particular attention to tests indicative of potential immunopathology and endocrinopathy (immune complex formation, tissue cross-reactive autoimmunity, hypersensitivity and hormone cross-reactive autoimmunity). Any and all other adverse signs detected, or symptoms reported, at examination and any abnormal findings in laboratory tests must also be fully documented and taken into account when deciding on the continuation or discontinuation of individual subjects or of the trial.

Subject and study discontinuation criteria

Participation in the Phase 1 trial will be discontinued for any subject who wishes to withdraw or who develops a significant study-associated condition which in the opinion of the investigator and/or sponsor of the study gives sufficient concern to warrant discontinuation. Similarly, the trial will be discontinued if two or more subjects develop a clinically unacceptable treatment-related condition. All subjects will be followed-up for the treatment of any study-related condition which leads to subject or study discontinuation.

Phase 2 clinical trial

Purpose

The principal purpose of a Phase 2 clinical trial of an antifertility vaccine is to determine the efficacy (i.e. prevention of pregnancy) of a selected dose of vaccine in healthy, fertile volunteers. In addition, the effects of giving at least one booster injection of the vaccine, as well as further studies of safety and the relationship between the subjects' genetic constitution and immune response, are also assessed.

Selection of trial centres

It is unlikely that one centre will be able to recruit all of the subjects required (see below) for a Phase 2 trial. It may be necessary, therefore, to identify two and possibly three centres at which the study can be carried out according to a common protocol. The facilities required at each centre will be the same as those for a Phase 1 trial.

Number of subjects

In order to determine the efficacy of an antifertility vaccine at a statisticallly significant level, compared to the known fertility rate of non-contraceptors in the study population, a total of between 750 and 1000 cycles of exposure need to be examined. The minimum number of cycles of theoretical protection against pregnancy provided by the vaccine can be deduced from the antibody

titre data obtained in the Phase 1 trial and, from this figure, the number of subjects required for the Phase 2 study can be readily calculated.

Selection of subjects

Subjects recruited for a Phase 2 trial of an antifertility vaccine will need to satisfy essentially the same inclusion and exclusion criteria as the Phase 1 trial volunteers. They will need to be healthy, fertile (but not pregnant or breast-feeding) and prepared to rely solely on the vaccine as the only method of fertility regulation during the exposure period of the trial.

Recruitment and informed consent

The means used to facilitate recruitment and the manner in which informed consent is obtained are the same for Phase 2 clinical trials as for Phase 1.

An important inclusion criterion is evidence of current fertility and at least three menstrual cycles should be monitored, therefore, to ensure that they are regular and that at least two of the three are ovulatory prior to admission. After immunization, cycles should be monitored, with at least one mid-luteal phase progesterone determination as an indication of ovulation. At entry into the trial, a full history and physical (including pelvic) examination should be performed on each subject together with cervical cytology, routine urinalysis, haematology, biochemistry and immunological tests. Certain of these investigations, together with an interim physical examination, should be repeated during the trial. A menstrual and coital diary and adverse reaction card should be maintained throughout the trial and frequent monitoring of the immune status must be maintained to ensure that pre-trial contraceptive practices can be reinstated when immmunity is estimated to be falling towards non-protective levels. The nature and timing of examinations and tests to be carried out in a proposed Phase 2 trial of a hCG vaccine are summarized in Table 16.2.

The same principles of informed consent that apply to Phase 1 trials will apply to Phase 2 with the additional need for the options available to the subject in the event of pregnancy occurring being fully explained to her.

Assessment of efficacy and safety

Studies of reproductive performance, including teratogenicity in relevant animal models, must be satisfactorily completed prior to initiating a Phase 2 trial of an antifertility vaccine.

A Phase 2 trial of an antifertility vaccine should involve the use of the optimal dose and administration schedule, or acceptable modifications thereof, as determined from the Phase 1 study. In principle, the number of subjects to be recruited to the study will be determined by the expected duration of efficacy, again estimated from the Phase 1 trial data, and the number of cycles of exposure needed to demonstrate a statistically valid antifertility effect. This would normally involve around 250 subjects studied in one or two centres.

Table 16.2. *Phase 2: Anti-hCG vaccine. Schedule of treatment and follow-up visits and procedures to be carried out at each visit*

Cycle phase:	Pre-injection cycles				Post-injection cycles																	
D1 = Day 1 MC = mid-cycle D8 = Day 8 ML = mid-luteal	Screening	Cycle 1 ML		Cycle 2 ML		Cycle 3 ML		Cycle 4 D8	Cycle 4 ML	Cycle 5 MC	Cycle 6 D1	Cycle 6 ML	Cycle 7 D1	Cycle 7 D8	Cycle 7 ML	Cycle 8 D1	Cycle 8 ML	Cycle 9 D1	Cycle 9 ML	Cycle 10+ D1	Cycle 10+ ML	Completion
Treatment (T) visits								T1		T2				T3								
Follow-up (F) visits									F1		F2	F3	F4	F5		F6	F7	F8	F9	F10	F11	
Injection								•		•				•								
HCG antibody	•									•	•	•	•	•		•	•	•	•	•	•	•
DT antibody	•									•	•	•	•	•		•	•	•	•	•	•	•
Progesterone			•		•		•		•	•		•		•			•		•		•	•
Full physical + Cx cytol	•																					•
Electrocardiogram	•																					•
Urinalysis	•									•		•		•			•		•		•	•
Urine microscopy	•													•								•
Haematology and HepBAg	•																					•
Biochemistry	•																					•
Igs and Complement	•																					•
RF and ANA	•																					•
Autoantibodies	•																					•
HIV	•																					•
DT skin test	•									•				•								•
HLA typing								•														
Interim physical										•				•								
Stop contraception											>----------											to cycle 11
Menstrual diary													(or) >			----------						to cycle 12
Adverse reaction card		>----------																				>
		>----------																				>

Note:
hCG, human chorionic gonadotrophin; DT, diphtheria toxoid; Cx cytol, cervical cytology; HepBAg, hepatitis B antigen; RF, rheumatoid factor; ANA, antinuclear antibody; HIV, human immunodeficiency virus; HLA, human lymphocyte antigen.

Coital records should be included in the analysis of the Phase 2 trial data to ensure that biases are not introduced into the efficacy rate as the result of changes in the subjects' behaviour between the pre-exposure and exposure phases of the trial. If legally and ethically acceptable, early termination of pregnancy should be offered as an option if pregnancies occur during the course of the study or in those subjects who maintain significant levels of immunity beyond the end of the study.

Although the principal objective of a Phase 2 trial of an antifertility vaccine is to assess efficacy, additional information on immunogenicity and side-effects is also obtained.

Subject and study discontinuation criteria

Participation in the study will be discontinued for any subject who:

(a) wishes to withdraw for any reason;
(b) fails to attain a satisfactory level of antibody;
(c) develops a significant study-associated condition;
(d) becomes pregnant.

The study itself will be discontinued if two or more subjects become pregnant with an antibody level at the peri-implantation stage of embryonic development that is above the estimated efficacy threshold, or if a significant number of the subjects develop clinically unacceptable treatment-related conditions.

Any pregnancies occurring during the course of the study and during the subsequent 3 years of post-injection surveillance will be carefully monitored. Offspring should be assessed, at delivery, at 12 months of age and at sexual maturity.

The same assessments that were carried out at entry into the trial will be carried out on discontinuation or at the end of the study. Subjects will be advised to return to IUCD use or barrier contraception at the end of the trial even if anti-hCG antibody titres remain at the theoretically effective level.

Phase 3 clinical trial

Purpose

The primary objectives of a Phase 3 clinical trial of antifertility vaccines are to determine the efficacy and safety of their use in the general population and in varied service settings. In addition, some indication of the acceptability of the vaccines will also be gained. A Phase 3 trial also offers the opportunity to assess the vaccine in a situation when it is given on a repeated basis, i.e. when booster immunizations are provided after initial immunity has waned.

Selection of trial centres

Several centres should be selected to represent a variety of economic and geographic situations, genetic or racial differences, nutritional status, and

types and levels of intercurrent infections. Some settings should mimic the potential field conditions in which the vaccine will be offered if it is approved for use in family planning programmes.

Number of subjects

At least 10 000 months of exposure should be studied, and this may require, depending on the duration of efficacy determined from the Phase 2 trial, in excess of 1000 subjects. Appropriate control groups should be included in Phase 3 studies.

Selection of subjects

Subjects will be recruited from the general population of fertile women requesting a family planning method and who are willing to use the vaccine as their sole method of fertility regulation.

Recruitment and informed consent

The means used to facilitate recruitment and the manner in which informed consent is obtained are the same for Phase 3 clinical trials as for Phase 1 and Phase 2.

Exclusion criteria

At entry into the trial, routine background laboratory, clinical and sociodemographic data will be obtained and subjects will not be eligible for a Phase 3 clinical trial of an antifertility vaccine if they:

(a) are pregnant;
(b) have an immunosuppressive or malignant disease;
(c) have a severe debilitating illness;
(d) have autoimmune disease.

Assessment of efficacy and safety

Phase 3 trials of antifertility vaccines will involve the study of 1000 or more subjects in different centres. Ideally, these trials should be randomized, controlled comparisons between the vaccine and an established family planning method. The studies should be designed with sufficient statistical power to demonstrate a clinically important difference between treatment groups, both with regard to efficacy and side-effects. It is difficult to choose a control method to be used in comparative studies with an antifertility vaccine, but logical choices might be IUCDs or barrier methods.

At a minimum, monitoring should comprise a menstrual diary and the keeping of an adverse reaction card.

Population differences may affect the results of large clinical trials with regard to efficacy and side-effects, and these differences must be allowed for in the design and subsequent analysis of the study. These considerations are particularly important for vaccines, where the immune response may be

modified by genetic variations, and by the influence of intercurrent endemic diseases. It is important, therefore, that Phase 3 trials are conducted in several representative populations.

Other factors of specific relevance to antifertility vaccine use require attention in Phase 3 trials, and in subsequent field trials, in which acceptability and utility are put to the test. These include, but are not restricted to, the following issues.

(a) The feasibility and acceptability of an antifertility vaccine if more than one administration is required for primary immunization.
(b) The need to analyze separately the data of lactating and non-lactating women in Phase 3 trials.
(c) The necessity to test for protection following primary immunization and as a guide to the need for and timing of booster immunizations. A requirement to obtain a venous blood sample for these purposes would severely limit the acceptability of the method. There is an urgent need to develop a simple and relatively non-invasive technique to assess immunity to the vaccine, preferably by self-monitoring. This could involve the use of a simple 'kit-type' colorimetric method of antibody measurement on a finger-prick blood sample or on saliva or vaginal secretions.
(d) In the absence of such a simple test, the alternative is to limit the time interval for boosting to the minimum period required for efficacy. This in turn would bear on the logistics of follow-up and return for booster immunizations, and the medium term effects of repeated secondary immunizations – say, over a period of 3 years. (Longer term studies would be the province of post-registration surveillance.)
(e) The need to monitor specific variables or phenomena related to the vaccine response. For example, if serological evidence of potential, but presumably inconsequential, cross-reactive autoimmunity arose in Phase 1 or 2 studies, this might require further examination by clinical review or functional tests in large numbers of users.

Subject and study discontinuation criteria

The study discontinuation criteria for a Phase 3 trial are the same as those for Phase 2.

The study will be discontinued if an unacceptably high pregnancy rate or a high incidence of clinically unacceptable treatment-related conditions occur.

Any pregnancies occurring during the course of the study and during the subsequent 3 years of post-injection surveillance will be carefully monitored. Offspring will be assessed, at delivery, at 12 months of age and at sexual maturity.

The same assessments that were carried out at entry into the trial will be carried out on discontinuation or at the end of the study. Subjects will be

advised to return to pre-trial method use at the end of the trial even if antibody titres remain at the theoretically effective level.

Ethical requirements

The ethical principles guiding the conduct of biomedical research involving human subjects are laid down in the Declaration of Helsinki of the World Medical Association as revised by the 29th World Medical Assembly in Tokyo in 1975 and in the CIOMS guidelines.[1] All clinical trials should conform to these principles and mention of such should be made in the consent form.

All information should be provided in both a comprehensive and a comprehensible manner, ideally in the form of a question and answer booklet that the subjects can read at their leisure and refer back to if necessary. Appropriate adjustment to, and allowances for, prevailing and individual literacy levels should be made in designing this document. Subjects must be given the opportunity to ask questions, which must be answered to their full satisfaction. If they then choose to participate in the trials, they should be asked to sign a consent form in the presence of a witness who also endorses the form.

If the subject's participation will in any way further research not directly related to the vaccine, this must be stated and explained and the subject given the option of withdrawing from that part of the study. If, after the subjects have agreed to participate, it becomes apparent that the use of their blood samples, other specimens, or data obtained from them would be useful for research other than that outlined in the original consent form, a separate consent form must be drawn up and signed by the participant permitting use of their data or specimens in the new research.

Above all, it must be remembered that, unlike therapeutic drugs that are used to treat specific diseases, family planning methods are intended for use by healthy individuals for prolonged periods of their reproductive life. This imparts an added dimension of ethical responsibility to the conduct of clinical trials of all new methods of fertility regulation including antifertility vaccines.

Legal issues

The legal rights and responsibilities of the trial subjects, the trial investigators, the centre at which the study is to be carried out and the sponsor of the trial, need to be clarified and safeguarded before the trial is initiated.

Long-term follow-up

Record keeping and administrative procedures should be established for all antifertility vaccines to facilitate long-term follow-up of subjects and selected offspring. This can best be achieved by setting up a central registry of data from all trials.

Long-term surveillance of antifertility vaccine use

The rationale for post-registration surveillance

The rationale for the long-term surveillance of subjects receiving antifertility vaccines is based on past experiences with drug and vaccine monitoring which suggest that many of the adverse effects of drugs or vaccines may only become detectable after a long period of exposure to the drug or immunogen. It is important, therefore, to undertake close surveillance for several years. In addition, adverse effects may be uncommon and therefore difficult to detect in the relatively small numbers of individuals involved in clinical trials which aim to detect side-effects that are reasonably common.

General guidelines and strategies regarding post-registration surveillance of drugs have been outlined in detail,[2–4] although there are two basic differences between therapeutic drugs and antifertility vaccines in terms of principles governing the assessment of safety and efficacy. Firstly, as has already been stated elsewhere in this report, antifertility vaccines will be used by relatively young, healthy people who have alternative methods of fertility regulation available to them. The safety of these vaccines, therefore, must be comparable to other family planning methods and probably higher than that of most other drugs in a therapeutic setting. Secondly, in terms of efficacy, with antifertility vaccines there is no equivalent to the herd immunity experienced with vaccination against infectious diseases, therefore antifertility vaccine failure rates need to be very low indeed, and again comparable to other available family planning methods if they are to find ready acceptance.

The long-term assessment of safety and efficacy of antifertility vaccines in the post-registration (post-marketing) era should include both the long-term follow-up of clinical trial subjects and the use of other approaches to detect adverse effects after the vaccine has been introduced into general populations. To date, the only relevant clinical trials to have been carried out are Phase 1 evaluations of various hCG vaccines. In the absence of extensive clinical trial data, it is difficult to be precise about all the parameters which should be monitored in the long-term follow-up of antifertility vaccine users and whether the considerations that apply to hCG vaccines will apply also to vaccines based on other immunogens.

The scope of post-registration surveillance

Although the majority of the subjects to be involved in long-term surveillance studies will be those receiving the vaccine at the post-registration stage, subjects involved in the pre-registration clinical trials will also be included as a valuable source of information generated over a longer period of time. The data to be collected and analyzed during post-registration surveillance of an antifertility vaccine will cover the following major factors.

Immunological characteristics of the target-oriented response

For example:

how many total courses of immunization can a woman safely tolerate?
what are the consequences of sustained, chronic immunity or of repeated immunization?

Endocrinological and other metabolic aberrations

For example, menstrual cycle irregularities.

Prejudgements and assumptions will be made on the basis of the above information concerning the number of courses of immunization that should be provided and the likely duration of efficacy that this treatment will afford in women, for example:

it is assumed that the number of courses of immunization may have to be limited, initially, to a maximum of three per subject until further information is generated;
it is predicted that with the advent of refined, controlled linear or pulsed release vaccine delivery systems (such as microspheres or capsules consisting of biocompatible and biodegradable materials), it would be possible to reduce the number of injections to one per course of immunization;
thus a single injection will be able to confer protection for a period of 12–18 months; and
the slow and controlled release of immunogen will reduce possible side-effects.

Assumptions, such as those indicated above, may have to be reviewed and modified periodically in the light of information generated from clinical trials. The necessarily small numbers of subjects involved in such trials (typically: Phase 1, 50 subjects; Phase 2, 200 subjects; Phase 3, 1000 subjects) will enable abnormal reactions, if any, to be detected at the level of 0.1–2% but will lack the statistical power to detect rarer clinical events. In order to pick up abnormalities at the level of, say, 1 in 5000 or so, a defined number of subjects, in excess of 30 000, may have to be recruited.

General approaches to long-term surveillance

In general, long-term surveillance would cover:

clinical and epidemiological observations on clinical trial populations and/or other vaccinated cohorts;
other epidemiological strategies in general populations using antifertility vaccines.

Specific strategies for clinical/epidemiological surveillance in a clinical trial setting and/or in studying cohorts of vaccinated women

In order for long-term follow-up of Phase 1, 2 and 3 clinical trial subjects to be carried out in the post-registration era, the following requirements will need to be met:

that the records collected during clinical trials are kept and are available for future use;
that means of keeping track of trial participants over long periods of time are considered;
that methods of follow-up are defined and subjects' consent is obtained for long-term follow-up;
that methods of reporting adverse reactions/disease/pregnancy need to be specified;
that consideration be given to the use of controls in clinical trials to facilitate the interpretation of disease occurrence.

Recruitment of additional clinical trial/cohort populations in the post-registration era may be required in order to increase the number of subjects under surveillance to 20 000–30 000.

Planning of both the practicalities and the statistics of long-term, post-registration surveillance will need detailed consideration in the light of evidence from Phase 3 trials and speculation about any possible remote side-effects of vaccination at the time of the completion of these trials.

Relevant factors in planning the study size are:

estimations of the rate of occurrence of illnesses/events relevant to surveillance in the population;
the increase in risk of the illnesses/events in the vaccinated population that is considered clinically important;
the level of statistical significance required and the statistical power required.

It is difficult to be precise, at this stage, about the duration of follow-up or the number of subjects required. These will depend, respectively, on the postulated latent period between vaccination and the development of any disease, and on the incidence of such disease. In addition, the extent to which particular conditions (e.g. long-term adverse effects on the offspring of previously vaccinated subjects) are studied will depend on the strength of the evidence that these conditions pose health problems.

Key methodological features of post-registration follow-up depend on:

whether it is possible to randomize subjects in Phase 3 trials to an immunization group and a group using other methods of contraception: and, if not,

the selection of cohorts or population-based information with which to compare the experience of disease in the vaccinated cohort.

The cohort study

In the absence of a randomized or other similar experimental design, the methodological features of follow-up of clinical trial populations and post-trial cohorts of vaccinated subjects are essentially the same. The cohort study offers a number of advantages over a case-control study for the post-registration identification of adverse effects that may be associated with the use of an antifertility vaccine.

(a) In a cohort study, groups of vaccinated subjects are followed up over time and their health status compared with unvaccinated cohorts or an appropriate section of the general population. This enables measurements of the incidence of diseases and reactions and of their absolute and relative risks in the two cohorts.
(b) In a prospective cohort study, information is available to facilitate deductions about the temporal sequence of events.
(c) The selection of comparison cohorts may be less subject to bias than the choice of controls in case-control studies.
(d) The cohort approach is not dependent on the subject's memory, in contrast to case-control studies.
(e) Cohort studies enable a wide range of outcomes following vaccination to be monitored.

The main disadvantages of cohort studies are that they may present substantial practical difficulties because, for example:

(a) large number of subjects are required for study;
(b) the subjects have to be followed for long periods and maintaining contact may be difficult;
(c) detailed approaches need to be considered to identify women lost to initial routine follow-up.

For the above reasons, the administrative infrastructure required is inevitably complex and such studies are generally very expensive.

Possible strategies for epidemiological surveillance in general populations in which an antifertility vaccine is used

A number of study designs and research strategies could be used for the epidemiological surveillance of antifertility vaccine users.

Systematic reporting of disease thought to be associated with the use of the vaccine

Historically, publication by clinicians of case reports of suspected adverse

drug and vaccine reactions has been one of the main ways that adverse effects have come to attention. The main advantages of the approach are that it requires virtually no formal organization, is cheap and can occur independently of population size or characteristics.

Its main limitations are:

that it depends on the availability of journals to carry such reports and the astuteness of clinicians (and journal editors);
that it is generally impossible to obtain accurate data on the incidence of the adverse effect unless more formal epidemiological studies are then undertaken;
that such reports rarely establish cause and effect relationships;
that there may be biases in reporting.

In spite of these limitations, historically, case reports have constituted an extremely important alerting mechanism for the detection of adverse effects of drugs and vaccines.

Systematic reporting of suspected adverse effects of vaccines

Many countries now have formal procedures under which clinicians are requested to submit reports of suspected adverse effects of drugs to a central agency. The advantages over non-systematic reporting are obvious but, of course, any formal reporting system requires the existence or establishment of an organizational infrastructure. A possible modification of reporting to a general-purpose drug/vaccine surveillance system would be the establishment of a reporting system specifically for antifertility vaccines, possibly including planned, registered release.[5] The major drawbacks of such a scheme would be those of additional organization and cost.

Investigation of clusters of disease hypothesized to be associated with vaccine use

An apparent clustering of disease, perhaps identified by clinical observation or reports or through routine health information systems (e.g. mortality records, cancer registers, congenital malformation registers), and its subsequent investigation is another non-systematic approach to identifying drug and vaccine side-effects which has been useful in the surveillance of adverse effects of drugs in the past.

Case-control studies

In a case-control study, a group of subjects is identified the members of which have a disease suspected to be associated with vaccine use. A similar sized, closely matched control group of subjects without the disease is also identified. The vaccination rates are compared to determine if there is a significant difference in the rate of vaccine use between the two groups.

The advantages of the case-control study are:

that it is useful for studying rare diseases;
that it is quick, cheap and easy to undertake compared with cohort studies.

The disadvantages of the case-control study are:

that it may be difficult to identify a truly appropriate control group and therefore interpretation may be difficult;
that it is not particularly useful for infrequent exposures (i.e. low population levels of vaccination);
that it is dependent on accurate memory of and/or records of vaccine exposure;
that it generally provides information on relative risk only (the absence of information on absolute risk and increased incidence makes it difficult to put findings in perspective);
that it only provides information about one disease per study.

Cross-sectional studies

In a cross-sectional study, people in the general population are identified for study to ascertain simultaneously:

their vaccination status; and
aspects of their health status relevant to issues of concern in the safety or efficacy of the vaccine.

Correlations between these parameters are then sought.
The principal advantages of a cross-sectional study are:

measurements of vaccination status and health status are made simultaneously;
such studies do not require the passage of long periods of time.

The disadvantages of a cross-sectional study are:

if the exposure (vaccination) and/or the parameters being measured as an index of health status are uncommon, large numbers of subjects will be required;
the study does not provide information on temporal relationships between vaccine use and disease or altered physiology and may therefore be difficult to interpret;
the study will not identify any patients who have died from a vaccine-related disease.

Indirect epidemiological correlational studies

These studies involve the analysis of data on disease incidence or mortality in relation to data on the use of vaccines in the same population. The aim is to assess whether changes in vaccine use correlate with changes in disease frequency, such that cause-and-effect deductions can be made. This type of study is generally regarded as the weakest of epidemiological approaches, for

various reasons, including the difficulty of making cause-and-effect deductions and its dependence on the use (and therefore the availability) of routine mortality and/or morbidity data. However, when such data are available, these studies are generally inexpensive to undertake.

Some practical considerations governing choice of approach to surveillance

Consideration must be given to the characteristics of the populations in which surveillance may be undertaken. For example, it is important that their social and cultural characteristics permit acceptance of medical care, clinical follow-up and possibly clinical investigation and that they have relatively low levels of mobility which facilitates long-term personal follow-up.

Consideration must also be given to characteristics of medical services available to such populations. Populations in which follow-up studies are to be conducted need to be covered by sufficient medical and paramedical services to ensure that relevant disease/clinical events are adequately identified. Study populations should have access to stable medical reference points (a particular clinic, hospital, regular doctor) when they are ill (or pregnant); and they should be covered by adequate medical record systems. To facilitate long-term surveillance of efficacy and physiological measurements, a means to identify and recall samples of subjects for study should exist. The possibility should be considered that subjects in antifertility vaccine trials – and perhaps non-trial subjects who subsequently use antifertility vaccines – should carry cards indicating their antifertility vaccine status so that their medical services can record/report medical events in such people. This implies choice of a population likely to be compliant in these respects.

In addition, consideration must be given to the nature and frequency of diseases or events likely to be of interest in post-registration surveillance. Different strategies will be needed:

to identify conditions which are common and those which are rare;
to identify disease in offspring as well as disease in the vaccine recipients;
to identify any unpredictable adverse effects.

Finally, consideration must be given to the organizational infrastructure required, and its cost, to undertake the favoured options for surveillance. It is likely that compromises will be required between what is desirable and what is feasible and affordable.

Summary comments on strategies for long-term clinical and epidemiological surveillance

The balance of advantage between different methods of post-registration surveillance will vary according to the incidence, predictability and likely

latent period of a postulated side-effect and to the organizational and financial means available to undertake different approaches to surveillance. For the detection of all but rare adverse events, long-term follow-up of a large cohort of vaccinated subjects, whose characteristics prior to the onset of any adverse effects have been well documented, must be the method of choice. Such a cohort study may be undertaken as a continuation of follow-up of subjects from Phase 3 trials (if sufficiently large in size) or by post-registration recruitment of subjects to vaccination cohorts. A major advantage of cohort studies is their large size and it is crucial that such studies be undertaken with careful consideration given to comparison groups so that the occurrence of disease/events in the vaccinated cohort can be compared with that in similar, unvaccinated people. If randomized controlled trials – to generate 'experimental' cohorts – are considered impractical or unethical, the remaining options are the assembly of observational cohorts of vaccinated subjects and of subjects using other methods of contraception or (less satisfactorily) comparisons with disease rates in the general population or a subset of it.

The detection of rare side-effects, if any, will probably remain dependent on other methods of surveillance – in particular, clinical reporting and/or systematic reporting to drug/vaccine monitoring agencies. While this information may be sufficient for generating initial hypotheses, it will be necessary to design case-control studies to investigate specific events in greater detail.

Long-term immunological surveillance

A simple, sensitive, specific, quantitative, reliable, rapid and inexpensive method for testing efficacy (or measuring relevant antibody titres) needs to be developed so that vaccine recipients can readily and reliably check (or have checked) their level of immunity in order to determine when a booster injection of vaccine or an alternative method of family planning is needed.

Since antifertility vaccines are likely to stimulate polyclonal B cell activation, a theoretical possibility exists for the generation of cross-reactive immunity to host tissues. A sample of subjects should be selected, therefore, to be screened for evidence of the delayed presence or persistence of organ-specific and non-organ-specific antibodies reacting with host antigens and tissues.

In addition, tests should be carried out for evidence of renal damage and proteinuria due to immune-complex deposition. Optionally, tests for immune complexes may be carried out. If so, at least two reliable tests should be used.

Complement levels (C'2, C'4 or total haemolytic activity) should also be measured.

The number of subjects to be screened should be between 1% and 2% of the immunized population. The time of screening should preferably be after the third course of immunization.

Immunogenetics and the immune response

The genotype of the vaccine recipient can:

influence the magnitude and duration of the desired immunity elicited by the vaccine;
determine the range and nature of undesired immunity elicited by the vaccine, for example specific and non-specific autoimmune reactions, and the possible subsequent development of autoimmune disease.

It would be advisable, therefore, to analyze the major histocompatibility (MHC) antigen profiles of individual subjects and different ethnic groups that prove to be low responders or non-responders to a given vaccine in order to determine if this genetically induced low or non-responsiveness can be overcome by modifying the vaccine formulation. The same analyses will also determine if particular types of autoimmunity occur in association with specific MHC profiles.

Long-term endocrinological surveillance

As the major targets of antifertility vaccines are reproductive hormones and other self or self-like antigens of the reproductive system, the long-term endocrinological follow-up of vaccine recipients should place particular emphasis on alterations in the levels of reproductive hormones and the consequences thereof in hormone-dependent organs and systems. The physician can be alerted to look for some of the adverse reactions which may be relatively easy to monitor, and the *modus operandi* for the assessment of the remaining parameters can be designed at a later stage.

Monitoring of the offspring

Children born to women during the post-immunization period should be closely followed until they reach sexual maturity for evidence of congenital malformations, abnormalities of secondary sex characteristics, and development of possible neoplasias which could directly be linked to hormonal manipulations.

Special considerations

Research should be encouraged with a view to determining what clinical conditions should be considered contraindications to the use of antifertility vaccines. For example: extensive skin allergy or atopy; overt autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, polyarteritis nodosa, etc. (antifertility vaccines might be contraindicated for this latter group for two reasons: such diseases could be exacerbated by the general

immunostimulation inherent in the administration of antifertility vaccines and/or antifertility vaccines may be ineffective because of immunosuppressive regimes these patients are likely to receive); hepatitis B infection and carrier states because of the inherent danger of developing active chronic liver disease; HIV positivity because of the risk of exacerbating the progression to AIDS as a result of T cell activation and facilitated viral replication (this issue has been addressed elsewhere in this report).

Social, ethical, legal and regulatory aspects of antifertility vaccines

The successful development and introduction of antifertility vaccines into family planning programmes is dependent on them satisfying a number of different but related requirements relevant to the eventual acceptability of this new technology by both the users and providers of birth control services. This section of the report addresses some of these issues and, as elsewhere in this report, uses hCG vaccines as an example, as they are likely to be the first antifertility vaccines to be used on a large scale clinically. It is anticipated, however, that the same issues will apply also to other antifertility vaccines based on alternative reproduction-specific antigens.

Social aspects

Of primary concern to the successful introduction and continued use of antifertility vaccines is that these new methods will be acceptable to the user. Acceptability will be influenced by a number of factors, such as the perceived mechanism of action, risk–benefit considerations, reversibility, adverse side-effects, and cost of the vaccines.

Mechanism of action

Prior to their introduction into family planning programmes, it will be important to determine, and disclose to the potential user, the known or suspected mechanism of action of each antifertility vaccine. In particular, there is a need to distinguish between those vaccines that prevent fertilization and those that prevent implantation. The relative acceptability of these two types of vaccine will vary from one location to the other as the result of religious and cultural differences as well as individual user preferences.

Risk–benefit considerations

The potential risks and benefits of antifertility vaccines need to be assessed both from the biomedical viewpoint as well as from the viewpoint of potential misuse and abuse.

Biomedical benefits of an antifertility vaccine include the following.

(a) Ease of administration by a procedure already associated with major health benefits. This benefit will be enhanced if effective levels of immunity can be elicited by a single injection.
(b) Potential long, but not permanent, duration of effect. This benefit will be enhanced if the period of efficacy is consistant and predictable.
(c) Potential suitability for birth-spacing. This depends on the return of fertility when immunity has waned to the non-effective level, for example, after 12–18 months.
(d) Potential high degree of efficacy. This would need to be comparable to other acceptable methods of family planning.
(e) Lack of endocrine disturbances and menstrual cycle irregularities.

Biomedical risks of the vaccine include the following.

(a) The production of autoantibodies and immune-complex formation.

Many antigens, and probably most vaccines in clinical use, induce the formation of antibodies that cross-react with normal body components. However, it is not known if and when this autoimmunity progresses to autoimmune disease or other pathological sequelae. The short- and long-term consequences of cross-reactivity must be carefully considered, therefore, particularly for antifertility vaccines, such as those based on the whole beta subunit of hCG, which are known to elicit cross-reacting antibodies, in this case to human luteinizing hormone (hLH). Although extensive cross-reactivity with hLH was reported in the Phase 1 clinical trials with these particular vaccines, there were few menstrual cycle disturbances, and ovulation appeared to be normal. The question of whether transient cross-reactive immunity resulting from the use of antifertility vaccines will lead to the development of autoimmune disease or other pathology remains to be resolved.

(b) Pregnancy occurring in the presence of immunity elicited by an antifertility vaccine. Risks to both the vaccine recipient and to the fetus need to be assessed in this context.

Past clinical trials of other contraceptives have shown that pregnancies do occasionally occur. In the case of antifertility vaccines, pregnancy may occur during the waxing and waning phases of immunity when antibodies are below the effective level, or immunization may be carried out during the early stages of an undetected pregnancy. It is important to determine if there is a risk of abortion occurring in any of these situations and, if so, when, so that the health consequences for the vaccine recipient can be assessed.

Although the potential for direct deleterious effects, both short and long term, on the development of the fetus and offspring is unknown, some tests in relevant primate species may be needed to determine if

the vaccine has the potential to induce teratological effects and to evaluate the reproductive capacity of offspring born to previously immunized mothers.

(c) Ineffectiveness of and/or disease exacerbation by the vaccine in women with a compromised immune system due to, for example, HIV infection, malnutrition, or parasitic diseases.

Abuse risks associated with antifertility vaccines are the same as those that apply to all methods of birth control, and include the following.

(a) The illegal use of the vaccine to induce an abortion.

Although vaccines directed against post-fertilization antigens, such as hCG vaccines, are not being developed as abortifacients – indeed pregnancy will be a contraindication to their use – there is a potential for abuse of these preparations if they are known to be capable of acting as abortifacients or are perceived to do so. The studies referred to previously [see (b) above] will be important in this regard.

(b) Administration of the vaccine without the consent or knowledge of the recipient.

In order to keep them distinct from other vaccines, such as those used in mass vaccination programmes against infectious diseases, it is important that antifertility vaccines are distributed through family planning programmes and services. However, in some countries family planning and vaccination services are provided by the same clinics, and often by the same individuals. Under these circumstances, it may prove difficult to ensure that antifertility vaccines are kept distinct from other vaccines and there is a risk that they may be administered incorrectly, as one of a group of antidisease vaccines.

In addition to the need to adhere to Good Manufacturing Practices in the production of antifertility vaccines, there is a need for 'good health care practices' to ensure that the risks indicated above are minimized or eliminated and that these vaccines are properly used. In view of the important role of the health care system in maximizing the availability, acceptability and benefits of antifertility vaccines for the user, studies should be carried out to determine the health care system requirements for these vaccines as well as the potential impact of the vaccines on health care delivery systems.

Reversibility

In contrast to antidisease vaccines, which are intended to achieve possible lifetime immunity following a single course of immunization, antifertility vaccines are designed to have a fixed duration of effect after which fertility will return. In order to be an attractive addition to the currently available options for family planning, it is assumed that the duration of effective immunity would need to be a minimum of 12 months and a maximum of 18–24 months.

Although major advances are being made in the field of vaccine engineering, which may make it possible to produce a range of vaccine formulations capable of eliciting different but predictable durations of effective immunity, the genetic constitution of the recipient is likely to remain a major influence on both the magnitude and duration of the immune response raised to each vaccine formulation. In some individuals, therefore, the immunity produced may be of a longer duration than intended or required. The acceptability of the vaccine to the consumer is likely to be greatly increased if safe methods to reverse the antifertility effect can be developed. The strategy adopted for this purpose would depend on the composition of the vaccine and its known or suspected mechanism of action.

Adverse side-effects

Information on the nature, frequency and severity of side-effects associated with the use of antifertility vaccines will become available during the course of Phase 1, 2 and 3 clinical trials. However, adverse side-effects which occur at a low frequency may not be detected until after registration of the vaccines. It is important, therefore, that the designated national authorities make post-marketing surveillance a condition for registration of the product. Such surveillance should cover adverse event reporting of biomedical parameters as well as reporting of consumer-perspectives and operational aspects of delivery of the method.

The kinetics of the immune response necessitate that users of antifertility vaccines maintain an overlap of previous contraceptive use during the rising and falling phases of immunity to ensure they are protected against the risk of pregnancy. Possible interactions could occur between the vaccine and other family planning methods, particularly steroidal preparations, to produce side-effects that might alter the safety and efficacy of the vaccine. This possibility should be considered when analyzing clinical trial data, particularly from Phase 3 and post-registration studies.

Cost

Although the majority of the research and development on antifertility vaccines is currently being supported by the public sector, a major financial and technical input from the private sector may be required for the large-scale manufacture of selected formulations.

Agreements must be drawn up, therefore, between public sector agencies and private sector manufacturers to ensure that these vaccines can be made available to the public sector in developing countries at the lowest possible cost. This can be effected either by provision of the product at a preferential price or by arranging for the transfer of the manufacturing technology to appropriate vaccine production facilities in developing countries.

In formulating these agreements, the following issues must be taken into account.

(a) Antifertility vaccines are being developed by the public sector agencies at the request of the governments of developing countries for eventual distribution through national family planning services.
(b) It is essential that the cost of antifertility vaccines to the public sector is kept as low as possible if these methods are to be viable options for the users and providers of these services in the developing countries.
(c) It is anticipated that if the vaccine is produced in large enough quantities, the costs can be comparable to those of the vaccines currently being used in mass vaccination programmes, for example the WHO/EPI programme of vaccination against certain childhood diseases.
(d) Sound patent coverage should be obtained and effective licensing arrangements made as the prospect of developing a significant private sector market in developed countries will favour the establishment of realistic international pricing practices and ensure the lowest possible cost of the product for the public sector in developing countries.

Ethical aspects

Informed consent

Ensuring that properly and fully informed consent is obtained from the potential participants is of primary importance in the clinical testing of antifertility vaccines.

Prior to registration, a detailed free and informed consent procedure needs to be developed and used consistently. The development of the consent procedure should involve user representatives to ensure that all relevant information is included in a comprehensive and comprehensible manner. It should describe the particular features of the new method and be appropriately updated as new information becomes available.

In determining the scope of the informed consent procedure to be followed when recruiting women for clinical trials, it is important that all appropriate information on known and possible effects is included. This issue has been addressed earlier in this report but can be summarized as follows.

(a) The possibility and consequences of developing cross-reactive autoimmunity.
(b) The risk of adverse effects to the fetus should pregnancy occur.
(c) The inability to provide absolute assurance that the effect of the vaccine can be reversed.
(d) The known or suspected mechanism of action of the vaccine. This should cover the immunological processes involved, including the potential of immunological disorders, and indicate the stage in the reproductive process at which the vaccine is believed to intervene. In the case of the hCG vaccine, the evidence from animal and human studies to date is that the vaccine operates after fertilization and before the end of the same menstrual cycle.

Collaborative studies

Phase 1 clinical trials of several hCG vaccine formulations are currently underway, or have recently been completed, under the auspices of three public sector agencies, and Phase 2 studies with these vaccines are now being planned. It is important that volunteers and investigators involved in future clinical trials have access to all relevant information from these studies to permit comparison of data relevant to the safety and efficacy of the different hCG vaccine formulations. To facilitate such comparisons, the use of common protocols in future clinical studies and in independent assessments of the immune responses elicited by the various vaccine formulations should be encouraged.

Legal aspects

An important factor in the decision to develop and manufacture antifertility vaccines is legal liability for the design, use and foreseeable misuse of the product. Manufacturers and distributors must anticipate having to meet legal liabilities to ultimate consumers and appropriate legal or institutional compensation schemes need to be developed.

Although the extent of liability will be governed, in principle, by national law, developed either through legislation or the decisions of authoritative courts, there is a growing trend in the field of products liability to adopt internationally agreed principles. However, the systems are far from being uniform, as evidenced by the following examples.

National laws may base liability on fault, so that where a party complaining of injury cannot prove fault on the part of the defendant, and also that such fault caused the injury complained of, the litigation will fail. Courts are increasingly ruling that a defendant's failure to make appropriate disclosures to the party suing is governed by the law of negligence. Where the party suing can show that had such disclosure been made, a decision not to use the product would have resulted, the failure to disclose will be held to have caused the injury to the user, even though the injury resulted from the irreducible risks of use of the product that no amount of care could have prevented.

In some legal systems the law provides for liability without fault where a product itself is considered inherently dangerous. Alternatively, courts may apply strict liability, meaning that the burden of disproving fault lies on the defendant. A principle of products liability law is that items may be marketed that are unavoidably unsafe (such as drugs) provided that they are not unreasonably dangerous. The courts will say whether a product is unreasonably dangerous, and whether liability is based on ordinary fault or on strict liability.

Manufacturers of antifertility vaccines, and those presenting such a method for testing, must determine the basis of liability within their jurisdiction and the jurisdiction of any country in which they propose that testing be conducted.

Liability may arise for such causes as:

(a) failure resulting in birth of a healthy child;
(b) birth of an impaired child due to product failure or improper administration;
(c) injury to the user.

A number of legal systems consider (a) compensible. Most would consider the extraordinary medical costs involved with (b) as compensible. The third case (c) would include such items as:

medical costs due to injury to the user;
out-of-pocket expenses such as lost income;
non-itemized general pain and suffering.

Some courts compensate all of these items, although the costs may be covered by commercial insurance.

Although the risk of such losses can be transferred to the user through adequately informed consent and the user's assumption of risk, this legal means of managing liability is ethically questionable. In clinical research, suppliers of a vaccine have a minimum obligation to undertake to provide medical services to users and children harmed through use of the product.

For wide-scale use of antifertility vaccines, studies need to be conducted of means of legally enforceable redress of any injuries that are found or are likely to have been caused by use of the product. Because recourse to litigation is unrealistic in many jurisdictions, alternative means of determining fact, establishing cause and assessing appropriate remedy or compensation will have to be established. There is a need to explore with governments means of prompt, effective and adequate relief for injured users. Governments should be encouraged to provide such a scheme of relief as a precondition to use of the product within their jurisdiction or by national agencies for use in other countries.

Two approaches can be considered for product liability coverage. Of primary concern is the need to respond appropriately when actual damage occurs to individuals receiving the product. Commercial manufacturers will generally be responsible for liability for marketing in the private sector.

(a) Liability protection for distributors could first be obtained through insurance companies. The insurance premium cost may have to be covered by an addition to the cost of the product. Although this surcharge could be significant initially, it is likely to decrease to modest levels as use of the vaccines increases.
(b) A further approach to liability insurance protection is to establish a self-insurance programme in parallel with commercial insurance. For each service or product provided, a small surcharge is added. Initially a portion of that surcharge is used to pay the costs of liability insurance, and a portion is used to establish a self-insurance fund. As the self-insurance fund increases in size, the amount of insurance purchased from commercial carriers can be reduced.

Consideration should be given to utilizing these and other approaches in connection with the distribution of antifertility vaccines.

There are two areas of biomedical research in which it is particularly difficult and expensive to obtain liability insurance protection, namely for the development of contraceptives and of vaccines. When the two areas are combined, in an antifertility vaccine, the problem is greatly compounded. There is, therefore, a major need for attention to be given to the issues of legal liability and compensation in respect of antifertility vaccines which, if unresolved, may hinder continuation of research in this important area.

Regulatory aspects

Although some progress has been achieved in establishing international requirements for the preclinical studies to be conducted, and the documentation to be submitted, in support of clinical trial requests, there are still major differences in the content, format and degree of detail that is required by different national regulatory authorities. The details of the information to be provided will depend on the type of antifertility vaccine under consideration; however, the following minimal requirements are likely to apply in all cases.

. *GLP/GMP requirements*

During the basic research phases of prototype antifertility vaccine development and safety assessment, Good Laboratory Practices (GLP) must be implemented.

The vaccine dosage forms to be used in the preclinical safety and efficacy studies and subsequent clinical trials must be prepared according to Good Manufacturing Practices (GMP).

Preclinical studies

The range and nature of preclinical studies to be carried out prior to each phase of the clinical evaluation of antifertility vaccines will depend on national regulatory requirements and legislation.

Prior to Phase 1

At this stage, safety and efficacy need to be demonstrated in relevant animal models. Safety evaluations should include (at least) acute and subacute toxicity in at least two mammalian species (usually mouse and rat), as well as muscle irritancy (in the case of vaccines administered intramuscularly) and pyrogenicity studies in the rabbit, and hyperimmunization (immunosafety) studies in a relevant infrahuman primate species. Efficacy evaluations must be carried out in a relevant animal model(s). The date obtained in these studies will form an essential part of an Investigational New Drug (IND) application.

Prior to Phase 2

Teratology studies need to be carried out in two mammalian species (usually rat and rabbit) and in a relevant infrahuman primate.

Prior to Phase 3 and prior to registration

The additional animal studies required at this stage of the clinical testing of antifertility vaccines are difficult to determine since they will depend on the particular vaccine under consideration and will be influenced by the nature and extent of the clinical data obtained in Phase 1 and 2 clinical trials.

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This, the fifth and final volume in the series on the scientific basis of fertility regulation, surveys the development and use of vaccines to control fertility. The volume arises from the World Health Organization initiative to develop new and improved methods of family planning. The book explores in depth the potential for harnessing the immune response to develop vaccines which inhibit fertility and which will provide a long-term but reversible form of fertility regulation. However, unlike most vaccines, which are based on 'foreign' antigens, antifertility vaccines need to be based on and directed against naturally occurring molecules essential for the reproductive process. This totally novel method of immunoprophylaxis has to be carefully controlled to avoid the danger of generating immune-mediated side effects. Safety aspects are therefore of paramount importance in the development of these vaccines and these issues are fully addressed in this volume.

The volume concludes with a discussion of the regulatory, legal and ethical aspects of this important new development.

The volume is suitable for all those with a professional interest in human fertility and its regulation: reproductive biologists, endocrinologists, obstetricians, gynaecologists, clinical immunologists and pathologists.

ISBN 0-521-39252-7

Cambridge University Press